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Studies in Pathology

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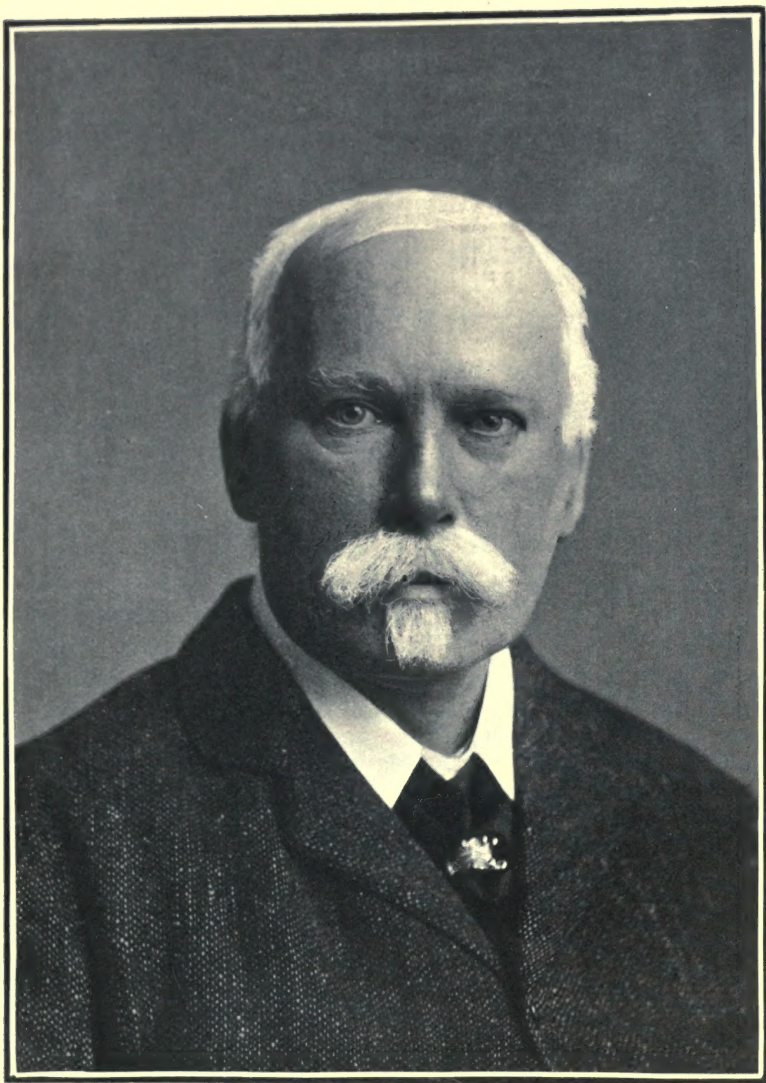
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H. Hamilton

Studies in Pathology

WRITTEN BY ALUMNI
TO CELEBRATE THE
QUATERCENTENARY
OF THE UNIVERSITY
OF ABERDEEN & THE
QUARTER-CENTENARY
OF THE CHAIR OF
PATHOLOGY THEREIN

Edited by

William Bulloch, M.D.

Bacteriologist to the London Hospital

ABERDEEN

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To
ALMA MATER.

To Thee we come again, bringing in our hands
these symbols of our Love and Reverence. . . .

We ask one thing only—that, at these splendid
Solemnities, we may lay our humble offerings on
the Altar, and, by this, renew our vows of Devotion
and Service.

P R E F A C E

SHORTLY after it was determined to celebrate the Four Hundredth Anniversary of Aberdeen University in 1906, the notion occurred to several of the medical graduates in London that a fitting tribute to the occasion would be the publication of a volume of original researches, in the domain of Pathology, written by those who had graduated since the foundation of the Chair of Pathology in 1882. The occasion seemed all the more appropriate for such commemoration, seeing that Professor Hamilton, under whose tuition the above graduates received their primary instruction, has all but completed a quarter of a century's tenure of office. A movement was started to sound our graduates holding appointments in Pathology as to how far they were in sympathy with the proposal, and the researches contained in this volume are the responses to that appeal, and are evidence of the feeling that prevailed among them, both for their Alma Mater and their teacher in Pathology. All the researches in the volume were specially written for it, and the cost of production has been defrayed by the contributors. It remains for the Editor to acknowledge, with deepest gratitude, the self-imposed labours of our University Librarian, Mr. P. J. Anderson, who, to all intents and purposes, passed the volume through the press.

W. B.

LONDON, *July, 1906.*

CONTENTS.

	PAGE
Professor Hamilton. By W. L. M.	xiii.
The History and Progress of the Sir Erasmus Wilson Chair of Pathology from the time of its Foundation to the present date. By W. B.	xix.
The Alimentary Canal as a Source of Contagion. By Professor Hamilton	(I)
A Remarkable Case of Bilharziosis. By William St. Clair Symmers, M.B., C.M. (1887) <i>(Illustrated)</i>	(39)
Malformations of the Bulbus Cordis. By Arthur Keith, M.B., C.M. (1888); M.D., F.R.C.S. <i>(Illustrated)</i>	(55)
The Administrative Aspects of Tuberculosis. By William Leslie Mackenzie, M.A., M.B., C.M. (1888); M.D., D.P.H. ...	(75)
Paroxysmal Irregularity of the Heart and Auricular Fibrillation. By Arthur Robertson Cushny, M.A., M.B., C.M. (1889); M.D.: and C. W. Edmunds <i>(Illustrated)</i>	(95)
Researches on certain Problems of Plague Immunity. By George Dean, M.A., M.B., C.M. (1889)	(111)
Experimental Study of the Immunity against Bacillus Pyocyaneus. By William Bulloch, M.B., C.M. (1890); M.D.	(157)
On Epignathus. By Alexander Low, M.A., M.B., C.M. (1896) <i>(Illustrated)</i>	(175)
A Contribution to the Pathology of Exophthalmic Goitre. By George Mellis Duncan, M.B., C.M. (1896) <i>(Illustrated)</i>	(199)
The Rat Theory of Plague Epidemics. By William Hunter, M.B., C.M. (1897) <i>(Illustrated)</i>	(215)

	PAGE
Some Experiments with Disinfectants. By Andrew Ross Laing, M.B., C.M. (1897); M.D., D.P.H.	(237)
On Eck's fistula—observations on Four Dogs, with a review of the literature relating to previous work on the subject. By John James Rickard Macleod, M.B., C.M. (1898) ...	(265)
On the Action of certain Bacteria in producing Cell-necrosis, with Special Reference to those of the <i>Bacillus Enteritidis</i> (Gärtner) Group. By George Ford Petrie, M.B., C.M. (1898); M.D.	(287)
The Relationship between the Factors inducing Haemolysis and those inducing the Phagocytosis of Red Blood Corpuscles. By Robert Donald Keith, M.A., M.B., C.M. (1902); M.D. (<i>Illustrated</i>)	(301)
An Experimental Enquiry into the Relationship of Leucocytosis to the Opsonic Content of the Blood Serum. By James Charles Grant Ledingham, M.A., B.Sc., M.B., C.M. (1902), and William Bulloch, M.B., C.M. (1890); M.D. (<i>Illus.</i>)	(321)
Immunity in Pneumococcal Infections. By George Grant Macdonald, M.A., M.B., C.M. (1903); M.D. (<i>Illustrated</i>)	(365)
Preliminary Note on the Bacteriology of some Diseases of Sheep. By James Milner Adams, M.A., M.B., C.M. (1905); and Bertie Ronald Gordon Russell, M.B., C.M. (1904) ...	(401)

PROFESSOR HAMILTON.

OVER twenty years ago—I remember it as yesterday—a wave of personal excitement passed over the Aberdeen Medical School. Nothing much had happened; it was only that the Students of the Fourth Year (in those days the last of the curriculum) had petitioned the University Court against including Pathology in the Final Professional Examination. Pathology, as a subject by itself, was then new; Professor Hamilton was then the new Professor. The students of Aberdeen, no more then than now, desired to shirk work; but it was the end of a long course not yet re-adapted, and many had begun it before a Pathology Chair was established. The dispute was very keen; it even affected old personal friendships; but the Court—it was in Professor Bain's first Rectorship—went with the students, and against the new Professor. Who raised the question, I never knew; but it made all the world talk of Pathology, and of its Professor.

Does any student in these luxuriant days know what went to the making of the splendid department he now works in? Will he look back with me and picture the hard beginnings of the New Prophet, the days in the wilderness, the triumphant personal energy that, day in, day out, unfaltering, unflinching, planned, and worked, and developed, and taught, until, like the pointed arch of a Cathedral, the new temple of a new science was reared, a fit sanctuary for our worship? It is not easy to

go in where a man's mind dwells, and live with him again the hopes, the disappointments, the despairs, the agonies that went to the creation of the vast fabric of Pathology as we now know it; but that is what every student of Professor Hamilton's would wish to do. The personal force that created his department created also the mind to work in it. This little group of studies is but a selection to mark the end of twenty-five years of working, teaching, stimulating, investigating, developing. There are worlds yet to conquer; but Professor Hamilton goes always forward. As twenty-five years ago, so now, he fights in the front rank; but he fights with the growing energies of all those years to aid him, with hundreds of old pupils living in his memory, with ever new generations of workers waiting for his voice, with opportunity, sympathy, a whole history of success.

Do you know or do you remember what Professor Hamilton was as a Teacher? I see him now come in—absorbed, active, energetic. He has undone his portfolio. He has opened his manuscript. He has glanced round to see that all his specimens are in their places. He looks up; he looks down; he puts his fingers into his vest pockets. He looks to the window on the right; he begins his morning parade. Then it is a drum-tap to attention. His fine baritone—trenchant, decisive, agreeable—gives the topic of the day. It was his method to select a definite idea, or part of an idea, to make it very precise in a condensed statement, to dictate this to his class until we all had it. Then he would analyze, illustrate, explain. He would show us this diagram, that specimen, this section, that section, until, when the hour was done, we had seen and understood and went away with a vivid impression vibrating in our memory. At the end, we crowded up to verify his points. He kept us close to actual specimens. We handled them, and he taught us how. We saw for ourselves. I have had twenty-six Professors, and among them many superb

teachers ; where so many were great, it is difficult to characterise, for each had his individual way, each his strength ; but Hamilton was among the most vivid, the most effective, the most stimulating.

And in his Practical Classes it was not different. Every leading point of his lectures and many more, he made real to us by naked-eye specimen, microscopic section, bacteriological culture. The pictures there seen for the first time are with me still—clear, defined, easily placed in the memory. Is there a better proof of good teaching ?

And in the Mortuary, the Teacher still predominated. He made the *post-mortem* room a new laboratory for the school. I knew the *post-mortem* room before Hamilton. How little we learned ! I knew it after Hamilton came. How little we missed ! He did not spare us ; neither did he spare himself. He drummed knowledge into us ; he commanded us not only to look, but to see ; he taught us method by making us act ; he taught us accuracy by making us weigh, measure and record ; he made us feel that science looks on nothing as unclean or common.

I remember, too, when the Text-book of Pathology was being forged. Every hour snatched from a busy day went to the searching of monographs, the verifying of references, the checking of theories. I am not a specialist in Pathology, and cannot tell how the book compares with the other great text-books ; but I should be surprised if it does not equal the best in lucidity and wealth of factual basis.

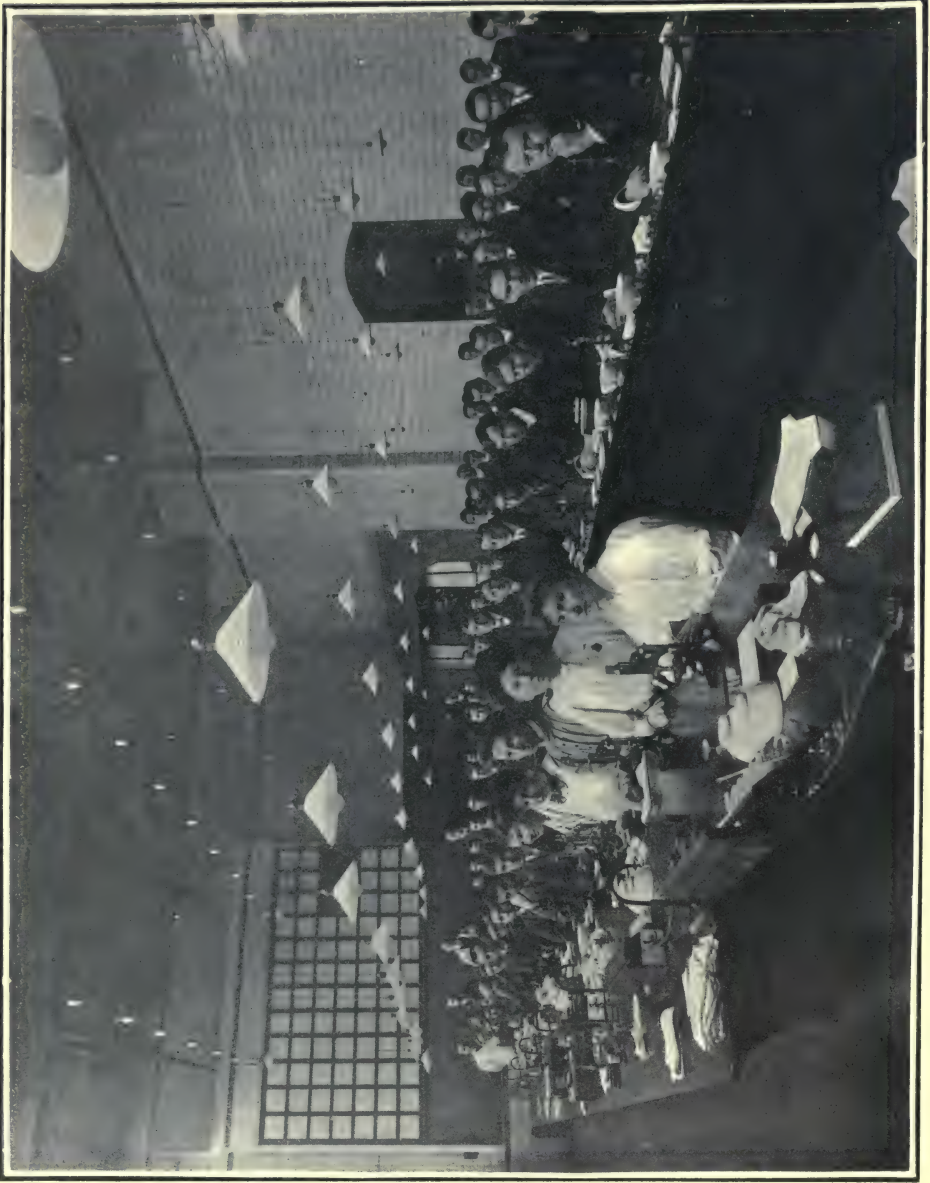
Of Professor Hamilton's work on the brain, his particular method of sectioning, his theory of the corpus callosum, and how many more points, I need say nothing. They were the furniture of our minds as students, and remain so. In the meanwhile he has worked along other lines. His splendid report, just issued, on Louping-ill and Braxy is one of the best

researches of recent Pathology. It is the result of many years of stringently scientific labour. It takes its place as one of the most solid achievements of the School.

But hitherto, I have been thinking of the strenuous, commanding, irresistible, earnest teacher of Pathology. It is all true; but it is not all the man. He is, too, an artist in music; he studies pictures and cathedrals; he sings, that in listening to him you forget the world; he lectures on Gothic, that you imagine here is his real love. And now I realise how much that voice stands for. If it was our trumpet-call on the field, it was, too, our sweet song in the moonlight, by the camp fires. Of all my twenty-six Professors, I hear the voices speaking still, and I know how little I have learned that did not come to me in those winged words. Some speak from the dead. But some are of the living. And we are going up for the Great Festival, where we shall hear them all again!

W. L. M.

August, 1906.



THE PRACTICAL CLASS-ROOM, PATHOLOGICAL DEPARTMENT, UNIVERSITY OF ABERDEEN.

The History and Progress of the Sir Erasmus Wilson
Chair of Pathology from the time of its
foundation to the present date.

FOR several years previous to the foundation of the Chair, it was felt that one of the main drawbacks to the reputation of the Aberdeen curriculum was the want of systematized teaching in Pathology. Many of the members of the Medical Faculty, and to their praise be it recorded, several of the oldest members, had long recognised this defect. The perfunctory manner in which the subject had been tagged on to the courses of Practice of Physic and Surgery, was, to say the least of it, unsatisfactory.

Doubtless a certain amount of instruction in morbid anatomy was to be had from the pathologist to the Royal Infirmary, but when one calls to remembrance the disadvantageous circumstances under which the casual demonstrations were conducted, the wonder is that they were attended even in the somewhat desultory manner which marked their career.

Germany and France were far in advance of Great Britain in this respect. They possessed, and had possessed for years, Chairs of Pathology not only in their large centres of medical education, but in every small University town, and were attracting all our best young graduates bent on acquiring the knowledge which was denied them in the schools of our

own country. Bacteriology had commenced to blossom forth as a new department of the subject—the year of foundation of the Aberdeen Chair will ever be memorable as that of the discovery of the tubercle bacillus. Physiology, moreover, had advanced to such an extent that an experimental pathologist such as Cohnheim or Stricker had become a possibility.

We were no worse off, however, than the Universities of Glasgow and St. Andrews. Edinburgh at that time was the only Scotch University, indeed the only University in the United Kingdom, which possessed a Chair of Pathology, and even the efficiency of this Chair had been for long stultified by its being occupied by one who, in theory as well as in practice, was a declared homeopath.

Opinion was thus ripe for the establishment of a Chair of Pathology in our midst, the only thing wanting being the money necessary for a foundation, and this was procured through the liberality of Sir Erasmus Wilson.

Erasmus Wilson was the son of Dr. Wilson, a native of the parish of Gartly, in Aberdeenshire. His father was in the King's navy, and was educated at Aberdeen University. While, however, his son Erasmus was still in his boyhood, he removed to London, in order to be nearer his ship, and Erasmus accordingly owed his medical education to the southern metropolis, having become a pupil of St. Bartholomew's Hospital in the year 1825, under Mr. Abernethy, with whom he soon became a special favourite.

It may be conjectured, however, that, like all true Scotsmen, Sir Erasmus had a warm side for his birthplace and scene of his early youth. Amidst the distractions of a large practice, and after the greater part of a life spent in London, he did not forget Aberdeenshire, and in a princely manner established the present Chair of Pathology by the gift of £10,000 to the University.

There were few in the medical profession of his day whose attainments presented such a varied aspect, and whose career was marked by such unusual success. Commencing life as an anatomist, Sir Erasmus did much, by writing and lecturing, to advance its claims, and to make its teaching easy. The *Dissector's Manual*, the *Vade Mecum*, and *Anatomical Plates* are evidence enough of the energy he developed in the study of the subject. In course of time, however, the founder of our Chair of Pathology renounced his anatomical pursuits to engage in the specialty—that of Dermatology—with which his name, medically, is chiefly associated, and in which he gained for himself a world-wide reputation. The works which he published on this subject, such as his *Diseases of the Skin*, his *On the Management of the Skin as a means of promoting and preserving Health*, his *Atlas of Portraits of Diseases of the Skin*, and many others, are well worthy to be, as they are, classical models for all time. In the year 1869, he founded the Chair and established the Museum of Dermatology in the London College of Surgeons, and was appointed its first Professor.

He was admitted a member of the Royal College of Surgeons of England in the year 1831, of which College he subsequently became an Honorary Fellow and ultimately President. In the year 1844, he was elected a Fellow of the Royal Society, and in that of 1881, the University of Aberdeen conferred on him its honorary degree of Doctor of Laws.

One of the subjects by which Sir Erasmus Wilson was best known, popularly, was that of Egyptian Archæology. We need hardly remind the reader of the works he published in this domain, and of the gift he conferred upon the nation by the transference of one of the Alexandrian obelisks to the banks of the Thames. He amassed an enormous fortune, partly from the practice of his profession, partly from judicious investment,

and out of this, besides his endowments in aid of medical education, he dispensed many gifts of charity, among them £30,000 to build the new wing of the Margate Royal Sea-Bathing Infirmary.

It is something for a man to leave money in bequests for educational and charitable purposes, but when it is gifted during the life of the donor it means a very great deal more, and as such the act demands correspondingly greater admiration. There is this, however, always to be remembered, namely, that gifts made during a lifetime for educational and charitable purposes, like the quality of mercy, are twice blessed; they not only benefit humanity at large, but reflect their reward on the donor through the pleasure afforded in watching the progress of the object they were intended to foster. Doubtless, it was a matter of gratification to the founder of the Chair to see a new subject safely launched upon the curriculum of one of our most ancient Universities.

It would be, however, a gross oversight in recording the history of the foundation of the Chair were one to omit mention of the part taken in the accomplishment of the deed by the late Professor Pirrie. He and Sir Erasmus Wilson had been close friends in their boyhood, and the intimacy and friendship had continued into years of maturity. No more ardent advocate for the establishment of a Chair of Pathology was to be found than the late eminent Professor of Surgery, and when the question of obtaining the foundation for such was being acutely discussed in Senatus and elsewhere, the happy thought occurred to him of making application to the friend of his youth. In answer to a letter which he addressed to Sir Erasmus Wilson he got back the reply that he (Sir Erasmus) never took any serious step in life without consulting two persons, namely, his lawyer and his wife. He saw that what was wanted was a matter of £10,000, and having obtained

the approval and assent of the above, he had pleasure in informing the Senatus that he was willing to give this sum to found the much needed professorship. He further added that he gave the money "as an expression of my regard for the institution in which my father—a native of Aberdeen—received his medical education, and as a recognition of the honour which the University has been pleased to confer on me by granting me the distinguished degree of LL.D."

The first Professor appointed to the Chair was its present occupant, Professor D. J. Hamilton. After several years occupied in Hospital practice in Scotland and England he gained the Sir Astley Cooper triennial prize of £300 for an essay on *The Diseases and Injuries of the Spinal Cord*, and thereafter resolved to devote his life to the subject of Pathology, although at that time there was but little promise of an opening in this Department of Medicine anywhere in Great Britain. With this view he proceeded abroad, and had the opportunity of studying with some of the most renowned pathologists of the day in Germany and France. He remembers seeing the venerable Rokitansky, the father of Pathology, sitting day by day in the *post-mortem* theatre of the Vienna Allgemeines Krankenhaus. He worked with Stricker for a winter upon experimental inflammation, and with Schenck on embryology, afterwards in Strassburg with von Recklinghausen, Waldeyer, Hoppe-Seyler and others, and subsequently with Virchow, Koch, and Pasteur.

He was thus enabled to adopt a system of teaching gleaned from the best models of the time, and to adjust this to the requirements of our own country.

On returning from the continent he was appointed Demonstrator of Pathology in the University of Edinburgh under the late Professor Sanders. This post he held for seven years before being advanced to the Chair in Aberdeen. While

Demonstrator in Edinburgh he was entrusted with the formation of a Class of Practical Morbid Anatomy and Pathological Histology. The class was perhaps the only one of the kind at the time in this country and, although purely voluntary, was attended by the whole of the students enrolled in the Systematic Class, and by many of those getting their education in the extra-mural school. He also succeeded to the office of Pathologist to the Edinburgh Royal Infirmary, on its becoming vacant shortly after he became Demonstrator in the University.

On taking up his duties in Aberdeen everything had to be organised from the commencement. Accommodation had to be found somewhere for the new classes of Systematic and Practical Pathology, a matter of no little difficulty in the then restricted space occupied by Marischal College. Through the acquiescence and kindness of the Professor of Medical Jurisprudence, Dr. Francis Ogston, his class-room was converted into a laboratory, and this for many years constituted practically the only premises belonging to the Chair. Here the class of students for years was instructed by relays in Practical Morbid Anatomy, Histology, and Bacteriology, while the Systematic Lectures were delivered in whatever class-room for the time being could admit of them.

Fortunately the newly appointed Professor had brought with him a sufficiency of material to start working upon, and with this he had to be content for some time to come. The Chair, unlike that in Edinburgh, did not possess any connection with the Infirmary, and hence the teaching in the one could not be associated with that in the other. In course of time, however, the pathologist to the Infirmary, Dr. Rodger, resigned office, and a strong appeal was addressed to the Managers to have the new Professor installed in his place, an appeal which was listened to and acted upon by them, so that the two Departments, that of the University Chair and that of the

post-mortem room in the Infirmary, came to be under the direction of the one teacher. The advantage of this to the students attending the class has been enormous, enabling them, as it has done, to connect the systematic teaching of the classroom with the invaluable instruction to be obtained in the *post-mortem* theatre. No Chair of Pathology can be completely efficient without this connection, for, in default of the practical experience to be had in the study of the effects of disease, as manifested after death, the teaching is always liable to become too strictly didactic, and to lapse into the domain of unproven theory. It has been the invariable endeavour of the present incumbent of the Chair to broaden the limits of the instruction afforded, by attacking the subject from many sides, and thus preventing his students from conceiving too narrow a scope of its compass. He has striven to connect the subject with physiology on the one hand and with clinical medicine on the other, and has strenuously opposed any tendency to specializing before an all-round comprehension of the possibilities of the subject had been acquired.

After several years, however, of probationary trial, the necessity of providing adequate accommodation, both at the Infirmary and at Marischal College, became apparent to the authorities concerned. A spacious *post-mortem* theatre was added on to the new Infirmary, and when the extension of Marischal College was taken seriously in hand, the building of a pathological Department was among one of the first practical outcomes of the movement.

With the advance of the recent discoveries in Bacteriology, it became highly desirable that arrangements should be made for teaching it both systematically and practically to each student taking the course, and this naturally required the necessary accommodation and teaching-plant. Provision was made, accordingly, in the new buildings to meet this requirement.

It is the opinion of the present occupant of the Chair, an opinion forced upon him by long experience and voiced by him on all available occasions, that the teaching of Pathology to be effective must be essentially practical, that although a certain amount of systematic instruction is absolutely necessary, yet, that the tuition which really drives the matter home and renders the knowledge gained practically indelible is to be acquired only in the laboratory. It is here that the truths brought before an audience of students in the class-room ought to be demonstrated and fixed in their minds, for by no other means is it possible to convince them that what has been described by the lecturer can be seen and verified by themselves. The combination of the two, in such wise that while the systematic description of the subject is fresh in their memories its truths are driven in upon their minds by actual demonstration, has always seemed to him the essence of all good teaching.

In planning the new Department, accordingly, it was his first care to stipulate for a teaching laboratory on such a scale as to admit of accommodating the whole class simultaneously, and in comfort.

Previous to the installation of the new buildings, it had been customary to give the Systematic Course in winter and the Practical Course in summer. This arrangement involved a great deal of overlapping, repetition, and consequent waste of time and energy. On application to the Senatus and University Court, however, permission was granted to combine the two courses of instruction in such a manner that, the systematic description of any subject having been given in the Lecture Room, the whole class, immediately afterwards, might be taken into the laboratory, and there made familiar practically with the object described. The teacher has thus been relieved from the monotony of delivering one hundred consecutive lectures, and

time has proved that the new system, from the students' point of view, is a decided advance upon the old.

The Department at the present time consists of a capacious lecture-room, practical class-room (see illustration), a museum, several bacteriological laboratories and apartments for special research, a photographic room, workshops, animal accommodation, and abundance of storage. It is quite possible that, before long, we may have to add on to these, but meanwhile they are fairly sufficient for the purposes required of them.

Mr. G. M. Duncan, M.B., C.M., Senior Assistant and Lecturer on Bacteriology, conducts a class of advanced Bacteriology, summer and winter, for medical graduates and those preparing for the Diploma of Public Health.

Dr. A. R. Laing, the second assistant, presides over the part of the municipal public health office concerned with the bacteriological examination of products from cases of contagious disease. The municipal authorities are in league with a large number of districts in the north-east of Scotland which are entitled to the same privileges as the city itself in so far as they can have brushings from diphtheria, typhoid blood, phthisical sputum, etc., examined on subscribing a merely nominal fee to the common fund.

One great object, all along, has been to encourage original research, and, since acquiring the new premises, such research work has been rendered possible. We have always a supply of young men anxious to do original work, and it is in this direction that it is desirable to extend the usefulness of the Department.

Even although the Department has been in existence for quarter of a century, it is yet far from being thoroughly equipped for either research or teaching purposes. Makeshift appliances have had to be utilized in the past which are not creditable to a great University, and which have seriously trammelled

the efficiency of the instruction capable of being imparted ; and even at the present day the working plant of the Department is anything but what it should be. It would require from £3000 to £4000 to put it on a thoroughly good working basis.

In addition to funds necessary for the complete equipment of the various laboratories, generous aid is also required in the endowment of research. There is no lack of young graduates who would be willing enough to undertake research studies provided we could furnish them with the means of living for a year or two. Our students are usually not overburdened with independent fortunes, and, consequently, as soon as they have graduated, most of them have to look out for some employment which will yield them the means of subsistence. Many of our most gifted young graduates are thus cut off from the chance of following the higher paths of medical science, and have to betake themselves to some obscure assistantship or other expedient for the purpose of earning a livelihood.

This seems a great reflection upon the efficiency of our University, and is one which is common to all the Scotch Universities. They have been content in the past with turning out graduates, quite oblivious of the fact that the mere imparting of knowledge to students is by no means the only function of a great centre of learning, indeed, is perhaps not the most important element in the work which should emanate from such. A great part of this teaching might be done quite efficiently by senior assistants or lecturers, leaving the Professor more leisure to conduct research-work himself, and personally supervise that of young graduates similarly engaged.

No doubt a little assistance in this direction has been obtained from various funds, manifestly from the Carnegie Grant to the Scotch Universities, but, with it all, the number of endowments in connection with these is limited, and the

restrictions set upon them are so stringent as to be almost prohibitive of their being accepted. When one thinks of the enormous sums of money which have been left for bursaries in time past, and which still come pouring in on all hands, when one considers the questionable efficiency of the subsidizing of students' fees by the immense sum of money comprised in the Carnegie Grant, several questions naturally assert themselves:—Has this money been laid out to the best advantage? is it subserving the purpose for which it was intended? and has it been directed into channels which are likely to yield the highest return in the future.

So long as Scotland, as in the 17th and 18th centuries, was a poor country, the good purpose subscribed by leaving money for bursaries could not be gainsaid. These bursaries in early times did an infinity of good in encouraging learning. Deserving merit through them met with its due reward, and was fostered and encouraged until such time as it blossomed forth in the cultured man of letters. But it becomes those who have the welfare of our Universities at heart, as well as those who are anxious about the education of the country in general, to ask themselves whether the system has not been overdone. Scotland is no longer a poor country; wealth has accumulated in it, and prosperity has followed the steps of those of her sons who have launched out in her various enterprises, indeed, to an extent which, reading the annals of a period so late as the end of the eighteenth century, could not have been anticipated by her most sanguine of prophets.

There is evidence, accordingly, that the subsidizing of the fees necessary for education at our Scotch Universities is in great part uncalled for at the present day, and, moreover, there is a suspicion abroad that it is actually doing harm by destroying the honourable feeling of independence and self-reliance which

has characterised the Scottish nation in the past, and which has been one of the mainsprings of her progress.

In face of this, think what might have been achieved with this money had it been applied to the proper equipment of our laboratories and the furnishing forth of our libraries. Both are in an inefficient state, and surely common sense would dictate that before announcing that we are prepared to turn out competent scholars, medical men, lawyers, etc., we should be supplied with the machinery for accomplishing this object.

We would enter an appeal in the strongest terms to benefactors of our University in the future, if they wish to confer a real benefit on higher education, to see to it that their benefaction is no longer cast into the educational slough known as the "Bursary Fund." Let it be applied to what are really the weak points in our system, namely, the equipment of our laboratories, the encouragement of research, and the endowment of our libraries.

W. B.

THE ALIMENTARY CANAL AS A SOURCE OF
CONTAGION.

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The Alimentary Canal as a Source of Contagion.

THE mucous membrane of the alimentary canal is pre-eminently an absorbent surface. It is constantly bathed in liquid swarming with bacteria, and yet only in rare instances apparently do these bacteria pass through its walls to gain entrance to the blood- and lymph-circulations. The great mass of them, many varieties being highly toxic, is rejected by the absorbents, and so, under natural circumstances, the blood remains free from contamination.

The cause of this cannot reside in the presence of a defensive barrier of phagocytes in the intestinal wall, for no such barrier has ever been demonstrated, and, moreover, it would be hard to suppose that any such means of protection would be sufficient to deal with the enormous numbers of bacteria of different kinds constantly passing along the intestinal channel.

Certain bacteria, as for example that of typhoid or of tubercle, do become absorbed, make their way into particular organs, and germinate upon them, while the numerous other organisms lying in their vicinity fail to be absorbed, or, if absorbed in small quantity, are destroyed by the blood or lymph.

How this is effected, and why there should be such a selective affinity in favour of one organism, is as yet shrouded in obscurity, although manifestly the function, whatever it may depend upon, must play an all important rôle in protecting the body under conditions of health from bacterial invasion.

Comparative Study of Certain Contagious Diseases of the Sheep.

The subject, it will be confessed, is one of intense interest, and my attention has been directed to it by the study of a remarkable class

of contagious diseases which affect the sheep. These diseases of the sheep seem to throw a great deal of side-light on the whole problem, and suggest that, likely enough, many diseases of man, whose pathology is still obscure, may be of intestinal origin, even where no such connection has been suspected.

The diseases in question have claimed my attention for many years past, first in a private capacity, thereafter under the auspices of the Highland and Agricultural Society of Scotland, and, lastly, since the year 1901, under the patronage of the Board of Agriculture and Fisheries. In the year 1901 the Board of Agriculture appointed a Departmental Committee to inquire into two of the diseases, namely, those known as Braxy (*Morbus subitarius ovis*) and Louping-ill (*Chorea paralytica ovis*), and our Report on these was issued a few months ago. It gave an account of the work so far as it had gone up to the date of publication (Report on the two Diseases of the Sheep, known as Braxy and Louping-ill. Board of Agriculture and Fisheries, 1906).

To this Report I must refer the reader for details, and meanwhile it is to the relationship existing between these diseases of the sheep and many obscure diseases of man that I would desire to enlist attention.

The sheep is peculiar in respect of the many contagious diseases to which it is liable, and it is curious that these heretofore have not claimed more attention than has been awarded to them. Thus, there is a large group the members of which are closely related in so far as they are each caused by a specific organism having certain mutual affinities and, apparently, of the same type as that of Tetanus. Several of the group have never up till now been recognised, and those whose characteristics have claimed attention have been investigated only in a perfunctory manner. Previous to the work of the Board of Agriculture Committee, little was known of most of them which could serve to explain their pathology and aetiology, or lead to their prevention.

So far as my own observations have demonstrated, the members of the group are comprised in the following:—Braxy (*Morbus subitarius ovis*), Louping-ill or Trembling (*Chorea paralytica ovis*), Malignant Œdema of the sheep, Blackquarter or Quarter Evil, the disease known as "Struck," and two diseases which, provisionally, I have named Disease "A" and Disease "B."

Some of them, such as Braxy, appear to be peculiar to the sheep, while others, such as Blackquarter, are common to it and to cattle. Louping-ill, although pre-eminently a disease of the sheep, is said to affect other animals, such as the calf, the pig, and the goose, but only on rare occasions.

Each of the diseases in question is caused by an anaërobic bacillus having a great tendency to spore, and whose natural habitat is the intestine. Doubtless they can all be inoculated upon the skin, and, when thus transferred, occasion a "Blackquarter" slough, but it is a mistake to suppose that even in the case of so-called "Blackquarter" this is the usual method of invasion. The "Blackquarter" slough is simply the manifestation of the effects of the action of the virus when implanted superficially upon a scratch or puncture wound, and can be called forth by any of them, while the commonest method of introduction, and this holds good of the whole class, is through the stomach and intestine.

Whether Malignant Œdema of the sheep is the same disease as that which affects other animals and man, and which goes by the same name, I am as yet unable to say, but quite possibly, from its occurring so frequently in the sheep, it is altogether a different malady.

There is another disease of the same class which is peculiar to the deer, but which as yet does not seem to have been identified or described.

Their Periodicity.—One remarkable feature of these diseases of the sheep and the deer is that they occur periodically, that is to say, at stated times of the year. Certain of them, such as Braxy, Disease "A," Disease "B," and Malignant Œdema prevail in the autumn and winter months, while others, and more particularly Louping-ill, are diseases of the spring; all of them tend to vanish during the summer. They show themselves, almost to a day, each in its season, and vanish quite as regularly and mysteriously.

Areas Affected.—They prevail only in certain districts, and mainly along the west coast and southern counties of Scotland and the northern counties of England, while the east coast of the whole of Great Britain may be said to be almost exempt from their ravages. Draw a straight line from the north of Scotland down to the south of England, and you practically separate the infected districts from the non-infected.

Braxy is a most destructive disease in Iceland, the Faröes, and the west coast of Norway; in fact it may be asserted that wherever the waters of the Gulf Stream impinge upon the littoral of a country, there Braxy will be found to prevail. It is quite likely that other diseases of the group infest such countries as well as Braxy, although nothing is known of the matter.

Pecuniary Loss.—The pecuniary loss entailed upon sheep-farming districts afflicted by these diseases, directly and indirectly, is enormous. It has been calculated that our loss in Great Britain must amount to something like half-a-million yearly, and this, I fear, is really an understatement of the case. So dreadful is the mortality in certain areas of Scotland that sheep farming as a profitable industry is ceasing to exist.

Chorea paralytica ovis, or Louping-ill.

It would be too large an undertaking to attempt even to outline the features of each of the diseases I have enumerated, in a communication of the present scope.

For the purpose I have in view, namely, that of illustrating the importance of the alimentary canal as a source of contagion in a number of diseases of man and the lower animals, whose pathology heretofore has been unexplained, I shall select one, namely, so-called "Louping-ill," that is to say, *the leaping disease*, a name applied to it popularly on account of the very manifest convulsive spasms with which the animal is affected. The late Principal Williams gave it the scientific designation of "*Chorea paralytica ovis*"—a term which seems to be singularly appropriate.

The disease, as just remarked, is one which prevails chiefly during the spring months, although sporadic cases occur in the autumn or early winter. The period extending from the middle of April to the middle of June may be said to mark the limits of its occurrence in an epidemic form, the middle of May constituting the zenith of its intensity. The valley of the North Tyne is one of the most severely smitten areas, and it was greatly through the interest shown in the matter by the Duke of Northumberland that the Board of Agriculture Inquiry was undertaken. The loss from it in the West Highlands of Scotland is tremendous, while all over the southern counties of Scotland its ravages

are well known. On the east of Scotland, however, from the extreme north down to the Lothians, the disease is so rare that some of those engaged in sheep farming hardly know what it is.

Symptomatology.—The symptoms can be divided into three distinct stages. In the first, the animal, as in so many of these contagious diseases of the sheep, is noticed to be somewhat dull. It may separate from the rest of the flock, stand apart in a listless fashion with drooping head, and be off its feed. It assumes subsequently a reeling gait as if intoxicated, and will lean against a dyke or fence for support. A dazed expression is often noticed in this stage as if the animal were in the initial stage of a fever.

These symptoms may last for a period of from two to three days, when it falls over quite unable to support itself or to regain, even temporarily, the upright position. The limbs are now spasmodically convulsed at intervals of perhaps a minute to a couple of minutes. The movements are mostly of a galloping character, and so incessant that the turf becomes worn in the area of their excursion. During the intervals, quivering or trembling movements are perceptible, hence the name "Trembling Disease" sometimes applied to it in the Western Highlands. The neck is drawn back as a rule, but the muscles of the neck are not intermittently contracted as in the case of the limbs. The temperature in this, the second stage, may go up to 105° to 108° F., and the pulse-rate and respirations are increased in number. The muscles of the jaws and those concerned in swallowing are not usually involved, nor is there any squint, and the intelligence of the animal appears to be little if at all impaired. It will nibble grass, and it swallows milk with avidity and without impediment.

There are cases, however, which assume quite a tetanic character. In these the muscles are in a state of rigid spasm, while, it is said, although I have never seen an instance of this in the natural disease, the muscles of mastication are in a like rigid condition, and in most respects the phenomena resemble those of idiopathic tetanus. Shepherds in Louping-ill districts will tell you of the occurrence of what they term "lockjaw" among their sheep during the spring months, and from the examination of the carcasses of those dying with such symptoms, and for other reasons, I have come to the conclusion that this disease is simply a severe variety of Louping-ill.

The animal may succumb in this second or convulsive stage, apparently from acute toxic poisoning. It passes into a semi-comatose state, the temperature sinks, and the convulsive spasms become weaker and weaker, preliminary to the fatal termination.

It should be mentioned that sometimes the animal suffers from diarrhœa with the presence of blood in the rectum.

Should the disease not prove fatal within a matter of a week or less, then there is every likelihood of its passing into the third stage characterised by the following phenomena :—The convulsive spasms of the limbs, so notable in the second stage, now give place to a condition of more or less complete motor paralysis; the limbs are outstretched and limp, while, if the sheep be held up by the fleece, they hang down relaxed and listless, and the animal is quite unable to use them for purposes of support. When the feet touch the ground the fetlocks are knuckled under in a perfectly helpless manner.

Painful sensation, so far as one can judge, does not seem to be affected to any appreciable extent even in this stage, but the reflexes from the limbs appear to be blunted, and in certain cases annulled.

The whole appearance of the animal closely resembles that of a person suffering from post-diphtheritic paralysis, the nature of the paralysis being essentially motor, and affecting the limbs by preference, although it seems to differ from the diphtheritic form in the fact of the palatal muscles being spared. During the course of the disease the animal is able to swallow liquid nourishment without impediment. The intelligence in this third or paretic stage often remains evidently uninfluenced. The animal recognises objects about it, and will bleat when a companion sheep is removed into a neighbouring pen. It will eat fodder if offered to it, but is quite helpless to seek for such of its own accord, and, as a consequence, in many instances, seems to die from starvation as much as from any other cause.

In this highly paretic state it may live for weeks, recovery seldom if ever taking place, even although the animal may have been fed artificially. Some subjects of the disease are said to make a partial recovery, probably only one limb remaining permanently crippled. I have always been somewhat doubtful, however, of the diagnosis in such cases; they look to me more like the effects of spinal abscess, which is very common, at least among lambs, during the spring months.

The following case will give an idea of the most striking clinical features during the first and second stages. The animal, a Cheviot ewe hogg, was procured from "clean" land in the year 1902, and took the disease accidentally on being transferred to our observation pens at Kielder. The first symptoms showed themselves on May 14th. On that day she was noticed to be a little dull, and was seen standing isolated on three or four occasions during the day.

On the morning of the 15th she was found to be duller than yesterday, and was the last sheep to rise this morning when fresh fodder was taken into the pen. She now stood completely isolated from her mates, and was not inclined to move with them. She turned round occasionally, slowly, and with restricted movements. The head was depressed, the ears failed to be pricked, and the eyelids were half closed. When standing, the animal's hind legs were somewhat flexed as if she were weak upon them, and when lying down all the limbs were gathered up towards the centre of the body. The rump was drooped, the back arched, and the front of the chest thrown forwards. She had not been seen to feed or ruminate to-day.

After standing for a few minutes, it was also observed that the animal became unsteady, and swayed a little from side to side, but quickly, and, as if conscious of the abnormal condition, she moved a few steps forwards to take up a fresh position. This, apparently, she did in order to prevent herself from falling forwards, and in the intervals stood in a drowsy, almost sleeping, attitude, the head gradually falling downwards with half-nodding movements, and inclined to the right side. A little rocking of the body from before backwards was evident, and in order to avoid pitching forwards she took a step or two. The animal, indeed, presented very much the appearance of being intoxicated. The whole body seemed to be in a slight quivering or shaking condition. The rectal temperature was 106° F., the pulse 80 per minute, and the respirations 80 and panting in character.

The animal was visited between 11 and 12 o'clock midnight, and was found evidently worse than during the day. She was lying against the wall of the pen, isolated, and did not rise when disturbed, nor did she offer any resistance when manipulated unless on two occasions, and at these times the body exhibited quivering or trembling movements. She could just manage to stand, and only for a short length of time; the gait was

very unsteady ; the free movements of the limbs were restricted, as if she was in fear of falling ; and the hind legs were occasionally snatched up spasmodically. When last seen on this night she was lying down quietly and looking the picture of dejection.

By the following morning (May 16th) the symptoms were more pronounced even than on the previous night. The sheep was now (between 8 and 9 o'clock) down on her side, and the most striking feature of the case was the occurrence of continuous violent convulsive spasms which persisted up till the time of death. The limbs were flexed and extended alternately, especially the posterior ones, which lay in a direct line behind the body. The movements of the fore limbs had a galloping character and were so continuous and violent that the underlying ground was worn out in a semi-circle from the friction caused by the excursion of the hoofs. The muscles were firm and rigid, and the contraction almost tetanic in character, although the tension was intermittent, not continuous as in tetanus. The head was thrown violently back and bent down on the dorsal spine ; it had also an inclination to the right side. The muscles of the neck were very tense during this action. The breathing was laboured, the animal gasping for breath during the height of an attack of convulsions, and, when there was a period of relaxation, it panted. Frequent and continuous nibbling or snapping movements of the mouth were noticed, and tufts of grass, or the soil, were occasionally clenched between the incisor teeth and the pad. There did not, however, appear to be any trismus. A puffing or choking noise was emitted through the nostrils, and frothy saliva began to accumulate in increasing bulk round the lips. During expiration the frothy saliva was projected in front of the animal for several inches to a foot.

The nostrils were widely dilated, but the breathing, although to a limited degree nasal, took place chiefly through the mouth, and was accompanied by guttural sounds. The muscles of the face, lips, and ears were in a tremulous condition, and during a lull between the crises of spasmodic attacks, the whole body was more or less tremulous. The eyelids were widely separated, and the pupils, if anything, somewhat contracted. The conjunctivæ were of a brick-red colour from injection of their blood-vessels, and the corneæ had now a dull appearance. The sphincter ani was contracted, and the perineal region was hot, wet, and clammy. The thermometer was inserted into the rectum with a good

deal of difficulty from resistance on the part of the animal. There was blood in the rectum to such an extent as to soil the thermometer, and the fingers were covered with blood after withdrawing the instrument. The tail, for the most part, was quite limp.

The abdomen was fully distended and tympanitic, and during an hour of more or less continuous spasmodic convulsions the distension visibly increased.

The external temperature was evidently high, and that within the rectum, at this time, was 108.5° F., the pulse 160, quick and short, and the heart could be plainly felt thumping against the thoracic wall.

When the legs were held in the palms of the hands above the hocks, they were stretched out rigidly, and tense quivering of the muscles was experienced during the manipulation. On seizing the limbs by the fetlocks they were snatched out of the hands violently and kicked out in a galloping excursion. When she was raised from the ground, the hind limbs were still extended backwards, became rigid, the hoofs were struck into the ground, and the body was thrown forwards, the animal resting on her knees and panting like a dog. If the support was withdrawn, the animal pitched forwards violently one or two yards and rolled over in a convulsive spasm. Placed upon her haunches, the spasmodic attacks still continued, the head was drawn back and to the right side, the fore limbs made the usual excursion in mid-air, and immediately the support was withdrawn she fell over in a convulsion. During the hour from 11:20 a.m. to 12:20 p.m., there were short periods of exhaustion and total collapse.

At 12:20 p.m. the convulsive seizures ceased, with the exception of a few rapid spasms of the fore limbs and occasional retraction of the head. The animal gave a long deep gasp or two and succumbed in what appeared to be a violent, almost tetanic, convulsion.

Morbid Anatomy.—On examining the carcase in this disease one of the most notable features is the absence of lesion which might serve to localise the peccant agent in any particular organ. Gas begins to develop in the abdomen very soon after death, and within a few hours, in certain instances, the wall of the abdomen may assume a greenish tint.

The abdominal cavity, as a rule, contains an excess of serous liquid, but this is not always the case. Sometimes the liquid is thick, muddy-looking, and, it may be, tinged with blood, while at other times it is quite

clear and limpid, or, at the most, a delicate coagulum separates from it. In no case have I seen peritonitis or pleurisy accompany the disease, and hence the conclusion seems inevitable that the organism which causes it is not possessed of inflammatory tendencies.

A few punctiform hæmorrhages may be met with along the course of the intestine, but with this exception all the viscera may seem to be quite healthy. Nor have I seen any evidence of meningitis or other disease of the central nervous system.

The microscopic examination of the natural liquids and of the organs of the body proves equally disappointing. Thus the blood is free from any micro-organism which can be detected microscopically, and when cultivated aërobically or anaërobically remains equally barren. The cerebro-spinal liquid and nerve centres are devoid of any parasite which might be taxed with a causal relationship to the disease, and, for these reasons, the pathology of the disease for long remained to us a problem fraught with obscurity.

During the first season (1902), in which we conducted our observations at Kielder, in Northumberland, we noticed, however, that two kinds of the case were brought in to us. In the one there was an excess of peritoneal liquid which was also turbid and sometimes slightly stained with blood. In the other the peritoneal liquid was perhaps not in excess, or, if so, it was quite clear and limpid.

On microscopic examination of the turbid liquid it was found to be teeming with a large coarse-looking rod-organism having a great tendency to spore, while in the case of that which was clear and limpid not a bacillus was to be detected. The rod in question had a close resemblance to that of Blackquarter, and, at first, we supposed that we had to do with two diseases running side by side, namely, Blackquarter and true Louping-ill.

On incubating the clear peritoneal liquid, however, in sealed tubes, I found invariably that, in the space of twenty-four hours, it became turbid, and when the tubes were opened, a whiff of gas escaped with a small explosion. On examination of the liquid microscopically, it was now found to be swarming with the same large sporing rod present in the liquid which was turbid.

This threw a new light on the whole pathology of the disease. There were evidently two forms, one in which the peritoneal liquid was

so full of a notable micro-organism that it could be readily detected by microscopic examination, the other in which the organism was so sparsely distributed in the liquid that it could not at first be detected microscopically, but in which the same specific rod developed abundantly on the liquid being incubated at a body temperature.

The turbid liquid was found in those animals which died within a few days, and with acute toxic symptoms, the clear liquid in those which lived longer and in which the disease went through all its three stages.

When the animal was slaughtered during the height of the malady the peritoneal liquid was always of the clear variety, the turbid variety was found only where the animal died a natural death.

Examination of the Peritoneal Liquid.—And here, perhaps, I may be allowed to emphasise the necessity for examining the peritoneal liquid in all diseases of a contagious nature. Casual reference is made to its examination in the literature on such affections, but it seems to me that the importance of examining it as a routine procedure has not been sufficiently recognised. The morbid agent in all these diseases of the sheep is invariably present in far greater quantity, and freer from contamination, within the peritoneum than in any other part of the body. The liquid can be drawn up in a pipette and sealed off with ease, and, so long as the organism contained in it is sporing, may be retained in an active condition for a matter of years. I have in my possession peritoneal liquid which is three to four years old, and which is just as potent to reproduce the particular malady when introduced subcutaneously, as it was when removed from the original host.

Description of the Organism.—The organism (*Bacillus choreæ paralyticæ*, Hamilton) possesses the following characteristics:—It is a large coarse-looking rod, sometimes elongating into a thread, or, it may be, a chain of rods. The actual measurements, as taken directly from the organism in the peritoneal liquid, I have found to be:—In one case, when not sporing, $5.6 \times 1.4\mu$, $7.0 \times 1.4\mu$; and, when sporing, $4.2 \times 1.4\mu$. In another case, when not sporing 2.8μ , 4.2μ , 5.6μ , 7μ , 9.8μ , and $11.2\mu \times 1.05\mu$ to 1.4μ . The spores in the latter case measured 1.05μ to 1.4μ in length. Its dimensions, therefore, like all the members of the group, vary considerably, chiefly accounted for by the fact that involution forms are almost always present. The ends are rounded, and it is

possessed of feeble motility. It has a considerable tendency to spore; the spore is located at its centre or at one end; and occasionally, especially after incubation in its native liquid, it assumes a drum-stick configuration, indistinguishable from that of the *Bacillus Tetani*. Most of the usual aniline dyes stain it readily, and the colour is not discharged by Gram's process. These staining reactions hold good of the organism, both when taken directly from the carcase and when in culture.

It is a strict anaërope, and grows on various media but most characteristically on alkaline glucose-beef-tea and glucose-gelatine, each covered with olive oil. The glucose-beef-tea becomes turbid after four to five hours' incubation at 38° C., and continues so for days while incubation is proceeding. If removed from the incubator after, say, four days' growth, the culture begins to settle down slowly at the bottom of the tube in a fine precipitate of greyish colour. It does not tend to agglomerate in a granular form, as in the case of *Braxy*, nor to become attached to the sides of the tube. During the process of germination much gas is evolved which possesses a distinctly putrefactive odour.

Examined microscopically, the culture is found to be composed of thick stout rods, somewhat longer possibly than in the original peritoneal liquid, slightly motile when first removed from the incubator, losing this characteristic later on. Their actual measurements were found to be:— $4.2 \times 1.4\mu$, $5.6 \times 1\mu$, $7.0 \times 1.4\mu$, and $14 \times 1.4\mu$.

The ends are again rounded, but usually the growth is free from spores. Even when the medium has been strongly alkaline to begin with, the *Braxy* organism will render it acid after a few hours' growth. The *Louping-ill* bacillus acts in a like fashion, but slower, and this probably accounts for germination being more protracted in the latter than in the former case.

Surface cultures on glucose agar grow luxuriantly; along the central streak formed by the inoculating wire, and from each side of this, somewhat arborescent processes extend outwards, rendering the sides of the central streak very irregular. Such cultures are not particularly diagnostic.

The stab-culture on glucose-gelatine, however, grown at 21° C., is very characteristic. In order to observe it, the gelatine should be covered with olive oil, and the inoculation made with the platinum wire through this. Quite a week will elapse before the growth reaches its best.

From the surface there passes downwards a grey-coloured streak for a

distance of a centimetre or so, but underneath this the culture becomes more expanded and often flattened out in a single lamella whose borders are occupied with a series of loop-like festoons. It liquifies the gelatine in course of time, but slowly, and when liquefaction around the culture is complete, the organism falls down in a greyish-coloured deposit. Gas-bells may or may not be liberated ; sometimes there is a single gas-bell at the deepest part.

Like the other members of the group, the culture in glucose-beef-tea bleaches dilute solutions of permanganate of potash.

The involution forms met with, as in the case of all the members of the group, are, at first, misleading. So varied may the general aspect of the organism be that it might be imagined that two or even three different bacteria were under observation, a fallacy which must be guarded against in arriving at a diagnosis of the case. The deception is particularly in evidence when Gram's process is applied to a culture on glucose-beef-tea. The organism invariably gives a positive reaction, but should such a culture be retained for a week or so at the ordinary temperature of a sitting-room, it will be found that the Gram's reaction is irregular, certain of the members giving an intense blue colour, while others remain only faintly discernible, or have a distinct pink tint. The pink tint is sometimes very remarkable, and forms a notable contrast to the intense blue of other bacilli lying in the neighbourhood. I have found this anomalous and irregular staining reaction in several members of this group of anaërobes, and do not consider it as peculiar to any one in particular ; not only does the colour differ, but those which have the pinkish tint appear very much more slender than those which give the ordinary deep blue colouration, so that, in an unguarded moment, one is apt to suppose that they represent two distinct bacilli. On examining the culture unstained, however, or stained with ordinary aniline dyes, the individuals will be found to be the same organism. The anomaly is evidently due to an optical effect consequent upon the one being intensely coloured, the other only faintly so.

Gram's process, as the literature on the subject proves in the case of Blackquarter, is extremely illusory when applied to these anaërobes, and requires to be practised with the greatest care. It will sometimes happen that the organism, taken from the peritoneal liquid directly, will give an

undoubted reaction, while, in culture, the same organism is completely decolorized. This I do not regard as a true reaction in any respect. If the organism be one which gives a true reaction, this should be forthcoming, both when it is growing in the native liquid, and when it has been cultivated on an artificial medium.

Inoculability.—When a small quantity, say half a cubic centimetre or less, of the peritoneal liquid containing spores is injected subcutaneously, and more surely if a few cubic centimetres of dilute acetic acid be injected side by side with it, the animal dies usually in from twenty to thirty-six hours afterwards. Some sheep are more resistant than others, but the experiment seldom fails to bring about a fatal issue, provided the animal be a one-year-old, and the experiment made during the months of prevalence of the disease.

I inject the liquid on the inner aspect of the thigh, as here the skin is free from wool and is cleaner than elsewhere. In a few hours after performing the operation the animal exhibits signs of distress. It has a dull expression, remains apart from its mates, ceases to feed or ruminate, and the inoculated limb is lame and has begun to swell. By a matter of from sixteen to twenty hours, these phenomena have become aggravated, the sheep has probably fallen over in a helpless state, may be delirious, and finally passes into a comatose condition for an hour or so before death.

At the autopsy the injected limb, sometimes indeed both limbs, and the front of the abdomen, are found intensely cedematous, so much so that occasionally the skin has ruptured and the liquid is escaping. The muscles for some distance round the point of inoculation are infiltrated with blood, and the areolar tissue is blown up with gas. The peritoneal liquid, as a rule, is abundant, opaque, and blood-stained, and, on microscopic examination, is seen to be literally swarming with the characteristic rod, which is sporing on all hands. The cedematous liquid of the inoculated limb also contains it, but not so abundantly as the liquid of the peritoneal cavity. It is usually absent from the heart's blood.

The organism is thus one of extreme virulence for the sheep, causing the death of the animal in a few hours after its introduction subcutaneously. It can also be inoculated upon the guinea-pig or rabbit, although apparently they are naturally immune to the disease.

Portal of Entrance.—The question of how, in the natural disease,

the organism gains entrance to the body of the sheep and to the peritoneal cavity, next engaged my attention. A common notion is prevalent among sheep-farmers that the tick has something to do with its transmission, but the theory is not supported by any well-grounded evidence founded on reliable observation. The tick, after hibernating upon rough dry scrub and rank grass, becomes parasitical upon the sheep during the month of April, and hence probably the origin of the supposed ætiological relationship. Suffice it to say, however, that, having sifted the matter very thoroughly, our Committee did not acquire evidence corroboratory of the allegation. Even although the organism may be present in the body of the tick, or, what is much more likely, merely adherent to its surface, the data at our command failed to prove that it is ejected from the rostrum into the skin of the sheep, and that the disease spreads from the tick-puncture as a centre. Many of the carcasses had not a single tick upon them, nor might there be evidence of a tick-bite. We never made out that local œdema, gas-formation, or hæmorrhage developed in the vicinity of the tick-bite, and in the case of an organism exciting such extreme local reaction as the bacillus of Louping-ill does, this seems strong *a priori* evidence against the theory of the transmission of the disease through the agency of the tick or any other animal parasite.

Nevertheless, if sheep taken from uninfected land be put upon that which is infected during the Louping-ill season, probably from a half to two-thirds, or it may be the whole of them, will be dead in from three weeks to a month afterwards.

The first case that really opened my eyes to the point of entrance of the organism was that of a lamb admitted to the Observation Station at Kielder during our second season, and in the year 1903.

A Cheviot lamb, perhaps about one month old, arrived at the Station at Kielder on May 14th, 1903.

The animal could walk, but in an uncertain swaying manner. The posterior limbs and hind part of the trunk gave way occasionally, and the animal fell on its side. The fore limbs and head maintained their natural posture, and even the hind quarters could in a manner be raised by an effort on the part of the animal, but soon again gave way. When leaning against a fence it would remain erect for a considerable time, seemed to be perfectly conscious so far as could be judged, did not

appear to suffer pain, and nibbled grass freely. When lying, the limbs were occasionally twitched backwards and forwards.

It remained in very much the same condition until the evening of May 24th, and was found dead on the following morning.

The examination was made probably from seven to eight hours after death. There was a complete absence of ticks, keds, or tick-bites, and the skin was perfectly sound and unbroken. The peritoneal cavity contained about 1 oz. of clear liquid, from which there separated a little flocculent albuminous precipitate. The liquid, however, did not coagulate universally on being retained in a sealed pipette.

On microscopic examination, a few colourless corpuscles were found within it containing very large granules, but nothing in the way of any micro-organism could be detected.

The carcase, otherwise, did not show any abnormality.

Anaërobic cultures were made from the contents of the small intestine. The tubes and their contents were heated up to 90° C. for a quarter of an hour, cooled down, and thereafter incubated. The cultures were started on May 25th, and by the following day were in a state of active germination, with tremendous evolution of gas. The medium employed was glucose-beef-tea. The organism which grew seemed to be quite pure, and consisted of a rod identical with that found in the peritoneal liquid in other cases, relatively thick, and free from spores. It did not appear to be motile.

The method of obtaining the material from the intestine for cultivation purposes was as follows:—The pieces of bowel, the small intestine, after being ligatured above and below, were cut off and placed in sterile Petri's dishes. The surface of the bowel was singed with a red-hot spatula. An opening was thereafter made with red-hot scissors and a platinum loop introduced right into the channel of the bowel. A little of the intestinal contents and mucus from the wall were withdrawn and mixed with the hot glucose-beef-tea. The wire was never allowed to touch the wound in the intestinal wall; the material was taken entirely from the interior.

After twenty-six hours' growth 4 c.c. of this culture from the intestine were inoculated subcutaneously into the left thigh of a Cheviot from "clean" land, along with 5 c.c. of a 1 to 10 glacial acetic acid solution in water. The inoculation was made on May 26th at 7.45 p.m.

By 8 a.m. on the 28th, the animal was noticed to be ill. It stood alone in the pen, pawed with its fore foot, and had, at times, an excited look. By 12 o'clock noon of the same day it was much worse. It lay down on its side and uttered a moaning sound as if delirious; its breathing was heavy. From time to time it arose, again to assume the recumbent posture within a minute or two. When lying down, the head was thrown straight out spasmodically and a little back.

By 4 p.m., or just about forty-four hours after receiving the injection, the animal died.

The autopsy was performed immediately afterwards. There was a complete absence of ticks. Both hind legs were intensely swollen from the presence of œdematous liquid, and, curiously, the uninoculated limb seemed to be more œdematous than that which had been the subject of experiment. The œdema also extended over the whole of the front of the abdomen. The liquid was serous in character, blood-stained, and was permeated with a bacillus identical with that introduced, slightly motile, and apparently not sporing.

The peritoneal sac was almost dry. The few drops of liquid removed were clear, and contained a good many lymph corpuscles, and a few bacilli.

The organs seemed to be unaltered.

A pipette was filled with the clear peritoneal liquid from the original lamb, sealed off, and incubated at 37° C. After twenty-four hours at this temperature, the contents of the tube had become thick and muddy. This result was due to a splendid sporing rod having developed within it, alike with that obtained from other animals dying of Louping-ill. The spore was oval-shaped, was placed usually at one end of the rod, and was of a faintly yellowish-brown colour. Many spores were also seen lying free. Certain of the rods did not contain a spore, and these were usually dividing.

About 2 c.c. of the incubated peritoneal liquid were mixed with about 10 c.c. of sterile distilled water, and 5 c.c. of the mixture were injected subcutaneously into the left thigh of a Cheviot hogg, side by side with 5 c.c. of a 1 to 10 solution of glacial acetic acid in water.

The experiment was made on May 26th, 1903, at 5 p.m., and by the following morning at 8 a.m. the animal was found to be very ill. It was lying down in the pen with the legs drawn under the body, and with a

tendency to somnolence. It did not rise when approached, and the breathing was rather hurried. At 5 p.m., exactly twenty-four hours after the commencement of the experiment, the animal died.

The autopsy was made immediately after death. The inoculated leg, as well as that on the opposite side, and the whole of the front of the abdomen, were intensely infiltrated with slightly opaque and somewhat blood-stained œdematous liquid. The œdema was extreme, perhaps greater than in any case previously observed.

Examined microscopically, this liquid was found to be swarming with a bacillus alike with that injected, dividing actively, but free from spores. It varied in length, some of the rods being short, others fairly elongated, and certain of them were distinctly motile.

This case, certainly, gave me an insight into the ætiology of the disease which previously was wanting. The animal was under our own observation when alive, the symptoms were typical of the disease, it died a natural death after about twelve hours' illness. Ticks and tick-bites were absent, and the carcass seemed to be free from disease so far as could be judged by mere naked-eye observation. The peritoneal liquid was in small quantity and was clear; it did not contain any organism detectable by microscopic examination. Anaërobic cultures of the intestinal contents developed pure growths of the Louping-ill organism, which, on being inoculated subcutaneously on a sheep, killed it within forty-four hours, its body permeated with the rod inoculated. The clear peritoneal liquid, after being incubated, became turbid, developed the Louping-ill organism, and this, on being injected subcutaneously into another sheep, killed it, in this case within twenty-four hours, the liquid of its tissues again swarming with the same organism.

Here is another observation having the same bearings:—

A four-year-old Cheviot ewe was admitted living to the Station at Kielder on May 29th, 1903. She had been ill for a considerable period, and had been lying helpless on the farm for a week. The symptoms were said to have been typical of Louping-ill, and by the time she reached us a state of almost total collapse had ensued.

The animal was found dead on the following morning (May 30th), and the examination of the carcass was made within a few hours of her demise. The abdominal wall in front had a faintly greenish tint. About

4 ozs. of liquid were found in the peritoneal sac. It was only slightly turbid, the turbidity being in great measure due to a flocculent precipitate, and was not blood-stained. On microscopic examination, however, most characteristic Louping-ill rods were found in it abundantly, some sporing, others elongating into threads.

On examining the contents of the small intestine, and more particularly the secretion on the surface of the mucosa, the same bacillus as that in the peritoneal liquid was found in tremendous abundance. It seemed, in fact, along with a little mucus and shed epithelium, to constitute the entire moist discharge lying on the mucous membrane.

The remaining viscera were somewhat congested, but otherwise not abnormal.

On May 30th, anaërobic cultures from the mucus of the bowel were made on glucose-beef-tea heated to 80° C. for twenty minutes, and were subsequently incubated. They grew luxuriantly, and by June 6th the culture had settled down at the bottom of the test-tube in the form of a dull grey-coloured deposit. Examined microscopically, the deposit was seen to consist of a rod identical with that of Louping-ill. It was comparatively thick, varied somewhat in length, and, at the time of examination at least, was not motile and did not appear to be sporing.

On June 3rd, 1903, 5 c.c. of this culture, which had been allowed to grow for forty-eight hours, were injected subcutaneously into the left thigh of a Cheviot hogg from "clean" land, side by side with 5 c.c. of a 1 to 10 solution of glacial acetic acid.

On the following day, the inoculated limb was considerably swollen; the animal was quite lame, and continued so up to June 6th, when it was slaughtered by bleeding. The object in doing so was, among other things, to ascertain whether the pure bacillus, without spores, introduced subcutaneously increases in virulence by transference from one sheep to another. The animal thus slaughtered had lived for three days after inoculation, and although very ill might have recovered.

The inoculated leg was found to be considerably swollen from subcutaneous œdema, and a minute and quite superficial abscess had formed at the point of inoculation. The whole of the inner aspect of the inoculated thigh and the front of the abdomen, together with the lower part of the inoculated leg, were very œdematous and blood-stained. The effused liquid contained the same bacillus as that injected, that from

the front of the abdomen possessing more of it than that from the leg. In the former situation, also, the rods were elongating, and seemed to be commencing to spore. There was about 1 oz. of peritoneal liquid, clear and limpid.

On June 6th, 1903, at 9 p.m., 1 c.c. of the liquid from the front of the abdomen was mixed with 8 c.c. sterile distilled water, and 5 c.c. of the mixture were injected subcutaneously into the left thigh of a Cheviot hogg from "clean" land, side by side with 5 c.c. of a 1 to 10 solution of glacial acetic acid.

June 7th, 8 a.m.—The animal was found to be lame in the inoculated leg, and had a somewhat collapsed appearance. By 10 a.m. it was much worse, and was leaning against the wall of the pen in a very depressed condition. Seen again at 11.30 a.m., it occupied exactly the same position, never having moved apparently since the last observation. At 9 p.m. the animal was prostrate and evidently dying. It was found dead on the following morning.

The appearances of the carcase were identical with those met with in similar experiments, only the œdematous effusion was very much greater than usual. It prevailed not only in the inoculated limb, ran out of it when the skin was incised, but also spread upwards all over the left side of the abdomen. The liquid was blood-stained and turbid, and contained the bacillus in great quantity. Some of the rods were sporing, others dividing. The œdematous subcutaneous tissue also showed some hæmorrhages and was blood-stained.

Briefly summarized, this case is to the effect that a four-year-old ewe was admitted to our Station at Kielder alive and suffering from Louping-ill. The carcase did not present any visible morbid feature, but the inner aspect of the intestinal wall, the mucous surface, was literally paved with the Louping-ill bacillus. This, cultivated anaërobically, grew with vigour, the growth consisting of Louping-ill rods, and when injected subcutaneously into a fresh sheep, caused great local reaction, œdema, and lameness of the inoculated limb. The animal was killed three days after being inoculated, and some of the œdematous liquid from the front of the abdomen was inoculated into a second sheep, killing it within something like twenty-four hours, the œdematous liquid again permeated with the specific rod.

These are samples of observations I have verified over and over again.

They require little comment ; they speak for themselves. The carcase in each case was free from evidence of disease which could be detected with unaided vision, yet, in both, the contents of the intestine were swarming with a specific bacillus which proved to be anaërobic, and which when inoculated into a fresh sheep rapidly killed it with evidence of intense local reaction. The disease germ, moreover, could be passed from host to host, evidently gaining in virulence by its transit.

The intestine the portal of entrance in the case of all the members of the group.—Not only in the case of Louping-ill, but in that of all the other members of the group the intestine seems to be the portal through which the organism gains entrance, and the fact that the peritoneal cavity contains it, more than any other cavity or any organ in the body, is thus readily enough explained. The peritoneal cavity is evidently the great lymph-sac of the body. Not only do its walls contain the lymph-vessels in connection with the intestine, but evidently those lymph-vessels returning from the hind limbs have also a free, although perhaps circuitous, connection with its interior. The organism of Louping-ill fails to propagate on the blood, but grows freely enough on the secretion of the peritoneal membrane, hence the large quantity of bacillus usually found in the liquid.

When the organism of the disease is inoculated subcutaneously in the sheep, death takes place so suddenly, evidently from acute toxic poisoning, that time and opportunity are not afforded for the development of the nervous phenomena. Where the organism, on the contrary, is introduced into the alimentary canal, and where the animal takes the disease but lives over, it may be, several weeks, the nervous symptoms are well developed.

Production of immunity to the disease.—It would be apart from the object of this communication to enter into the explanation of the production of immunity to the disease too minutely, and the reader is again referred to the Board of Agriculture Report (*loc. cit.*), already mentioned, for a full statement of the case.

The bacillus of Louping-ill can be administered to sheep by the mouth with impunity throughout the greater part of the year. As the susceptible months are approached, however, namely, March, April, and May, the danger of doing so is extreme, and a fair proportion of the animals so treated will die with all the classical symptoms. At other

times of the year the organism may and does pass along the intestine of the sheep without exerting any harmful manifestation. Indeed, at these times, it exerts a most beneficial influence in rendering the animal immune. My whole method of preventive treatment, as will be seen from the Report, is founded upon this principle, namely, the administration of the organism by the mouth at a time of year when the sheep is not susceptible to the disease. The organism multiplies in the intestine, but apparently, at these times, is prevented by some means from crossing the barrier afforded by the intestinal wall, and so does not find access to the peritoneal cavity. Nevertheless, it undoubtedly immunizes the animal and protects it from an attack of the natural malady. Out of a total of 1340 sheep treated by us according to this method during the year 1904-5, in the very worst districts of Scotland, and where we often shifted the animals deliberately from "clean" to "foul" pasture, we had not a single death from Louping-ill. A culture was administered to the animals mostly during the month of January, and not in a single instance did we find that it had, when administered thus early in the year, a baneful influence, and yet, nevertheless, it acted as a most effectual protective against the natural disease.

The subject of immunization through the intestine in the case of contagious diseases of man which are of intestinal origin has not, it seems to me, met with that attention which its importance claims.

How the immunization in the sheep is effected, I will not at present venture to explain. It may be that the epithelium of the intestinal mucosa becomes resistant to the passage of the organism, and thus prevents it gaining access to the peritoneal cavity, or it may be otherwise. To elucidate the problem will require much patient investigation.

Strangers visiting a foreign country and residing in towns where typhoid prevails endemically, are more likely to contract the disease than the regular inhabitants. May the explanation of this not be that the latter are habitually drinking typhoid, and have become immune to the fever without actually suffering from the malady? We know, as a fact, that immunity to the intensely poisonous substances ricin and abrin may be brought about by administering graduated doses by the mouth. It seems, therefore, rational enough to suppose that in the case of several diseases of man, especially those in which the intestine is primarily concerned, a like immunity may be established through the alimentary canal.

Whether the dead bacillus given in this way has a like effect I have not as yet determined, but I think it probable that, if employed in sufficient quantity, such may be the case. The immunizing principle is evidently contained in the protoplasm of the bacilli, and there does not seem any very evident reason for believing that it may not exert its beneficial influence if administered by the mouth, and in a condition fit to be absorbed.

During the months in which the disease is rampant, however, the protective influence of the intestinal wall is lost in a large proportion of cases, the organism gets over into the peritoneal sac, fructifies within it, and kills the animal.

The spores being voided with the dejecta are taken up from the pasture by a fresh host, and the result seems to depend very much on the season of the year at which this happens. Should it occur during the susceptible months the danger is extreme, while, at other periods, it is practically nil. Nevertheless, the younger the animal the more liable is it to the disease, and hence we may suppose that the ingestion of the organism at an early period must have effected its immunization.

Cause of periodicity of the disease.—The insusceptibility of the sheep to the disease at certain times of the year seems to depend directly or indirectly on the condition of its blood. The blood of the sheep during the spring months of the year usually constitutes an excellent medium of culture, while at other times it, as a rule, is not only inimical to the growth of the bacillus of Louping-ill, but is intensely bacteriolytic to it. So that if, say, the blood of the sheep during the month of July, be mixed *in vitro* with a culture of the bacillus, the mixture covered with olive oil, and the whole incubated at a body temperature for twenty-four hours, probably every bacillus will be found to have vanished. During the susceptible months, however, the organism multiplies and spores on the blood of the sheep perhaps better than upon any other medium. It is evidently this inhibitive action on the part of the blood during most of the year which prevents the organism growing upon it or upon the peritoneal liquid, for there is no reason for believing that the organism gets into the alimentary canal with more facility during the months in which the sheep is susceptible than at other times. The peritoneal liquid apparently does not possess solvent powers to anything like the same extent as the blood, and where the bactericidal action of the

blood is lessened as during the months of susceptibility, the organism is enabled to pass the wall of the intestine, to fructify on the peritoneal liquid, and to kill the animal acutely with all the symptoms of toxic poisoning. The peritoneal liquid in such instances is thick and turbid, and contains the organism in abundance.

Absence of Phagocytosis.—And here it may be mentioned that the destruction of the bacillus by the blood occurs quite independently of phagocytosis, for neither in the animal's blood nor in the peritoneal liquid have I ever seen evidence of phagocytosis in this or any other of these diseases. When, moreover, the organism is mixed with freshly drawn sheep's blood and incubated, not a bacillus will be found to have been inglobed by the phagocytes. Should there be, as there sometimes is, a coccus or other impurity in the blood, the phagocytes may be found to be packed with the impurity while not a single one of the specific bacilli has been seized upon. Whether this depends upon a powerful negative chemotaxis existing between the specific bacillus and the phagocyte or not is hard to say, but of the absence of the phenomenon there cannot be a shadow of a doubt.

Cause of the Toxic Phenomena.—It may happen, however, that in other instances the blood still retains sufficient bacteriolytic properties to dissolve any of the organism which gets into it, although it is not sufficiently inhibitive to prevent a certain exodus of the bacillus from the intestine. Those bacilli which enter the blood stream, under these circumstances, are still bacteriolysed, and apparently the same thing happens, but to a minor extent, within the peritoneum. The blood in such animals will be found free from bacillus and the peritoneal liquid may be clear and limpid, and not show any of the organism until after being incubated. These cases run a chronic course, and in them the nervous phenomena are most marked. The difference between the acute and the chronic case seems to depend upon the rapidity and volume with which the bacillus gets through the intestinal wall. In the chronic case the number is evidently small, and can be dealt with by the solvent action of the blood, while in the acute case the number is so great that the animal dies from rapid toxic poisoning. All seems to depend upon the condition of the blood. If it be in its usual state of antagonism to the growth of the bacillus, such as prevails during the greater part of the year, apparently the bacillus cannot leave the

channel of the bowel. If, on the other hand, this salutary property be weakened but not annulled, a certain leakage as it were takes place, but still the number of bacteria which finds entrance to the blood-stream or peritoneal cavity is small, and they can be got rid of by the bacteriolytic action of the blood-plasma or peritoneal liquid respectively.

Through the toxins set free from the bacteriolysed organism, in the latter case, a condition of chronic toxic poisoning of the animal ensues. If, again, the blood-plasma have lost its bacteriolytic, protective, action completely, the organism not only passes the barrier constituted by the wall of the bowel, but fructifies on the peritoneal liquid, killing the animal with symptoms of acute toxic poisoning.

It would thus appear that the sheep is a very remarkable animal in respect of its blood being highly protective at certain times of the year, while at other times this protective influence is more or less completely lost.

Whether such a relationship exists in a modified form in the human blood with regard to certain pathogenic bacteria may be a matter of question. No one has inquired into the subject, but, judging from analogy, there seems little reason to suppose that such a remarkable quality occurring in one mammal is not at least represented in another. The fall of the leaf and the spring of the year have always been held to be seasons of great susceptibility to certain contagious and infectious diseases. May it not be that the natural bactericidal properties of the blood, as in the case of the sheep, are lessened at these particular seasons? various pathogenic organisms being thus encouraged to grow upon the tissues or it may be upon the surface of the various mucous membranes.

The Toxines bound up with the Bacillus.—The essentially toxic substance of the Louping-ill bacillus seems to be bound up with the bacillus itself, and not to be shed into the liquid of culture. When a culture of the bacillus is made upon glucose-beef-tea, the culture filtered, and the filtrate injected subcutaneously in large quantity and on several occasions into the sheep, the operation does not call forth any symptoms; the sheep seems to remain quite unaltered in its condition.

Seeing that the symptoms are apparently caused by the solution of the bacilli, which find their way into the blood or lymph from the intestine, and whose toxins are thus liberated, it occurred to me that

were a large quantity of the bacillus bacteriolysed by the blood of the sheep *in vitro*, thereafter filtered, and the filtrate injected subcutaneously, I would be enabled to get the toxine in a soluble condition and to study its effects without the complications arising from having the bacillus itself to deal with.

The following experiment showed that the filtrate thus obtained proved intensely poisonous to the sheep, and induced symptoms which were identical with those of the severest forms of tetanus:—

A 300 c.c. flask of glucose-beef-tea was inoculated anaërobically with Louping-ill peritoneal liquid taken from an experimental sheep which died from the disease on February 20th, 1904. The culture was allowed to grow for forty-eight hours, and thereafter the supernatant liquid was decanted off and the deposit of bacillus mixed with sheep's serum containing a large percentage of blood corpuscles, as many indeed as could be procured by shaking forcibly the bottle in which the blood was contained. The mixture was covered with sterile olive oil, and placed in the incubator. The blood was obtained from an Aberdeen slaughter-house.

In the course of twenty-four hours a large number, but not the whole, of the bacilli had been bacteriolysed. Only a few were left, and many of these seemed to be in a state of impending dissolution. The coloured blood corpuscles were still retained in perfect preservation.

The mixture was passed through a Berkefeld filter, the liquid which came through being quite clear, and, so far as could be ascertained by microscopic examination, free from any bacillus.

On July 16th, 1904, 4 c.c. of the clear filtrate were injected into each thigh of a "Cross" one year old, obtained in the Aberdeenshire market. On the following day, and up to July 28th, nothing was perceived in the condition of the animal worthy of note. On that day it was taken very ill, and on visiting it at 5.30 p.m. it was found to be lying on its side in a perfectly helpless condition, the legs all extended and uplifted, and in a state of extreme rigidity. They could be bent, but with difficulty. The jaw was clenched, but at this period not tightly; the animal's intelligence seemed to be retained. The neck was stiff and drawn backwards, the eye clear. From time to time there was spasmodic contraction of the limbs, particularly if the animal was approached rapidly, or if disturbed by a sudden noise, as on walking over the stall.

There was not the slightest evidence of its suffering from diarrhœa, the neighbourhood of the tail being perfectly clean. The case at this stage resembled closely certain examples of natural Louping-ill with a tetanic tendency.

On asking the attendant what the first indications were pointing to the animal being ill, he said that, when making his usual visit on the morning of the 28th, it was standing with its head towards him, looking out between the rails of the pen. On approaching the pen, the animal walked across it, but in a very unsteady manner, and as if unable to maintain its balance. It did not seem to be convulsed; the tendency, he said, was rather to maintain the limbs in an outstretched attitude.

July 29th, 10 p.m.—The animal was much worse; it was now in a state of universal tetanic spasm. The limbs were all outstretched, and the muscles hard and rigid from tetanic contraction. The jaw was so firmly clenched that the mouth could not be opened to administer nourishment. Slight convulsive spasms were noticed from time to time, but the tonic state of contraction of the limbs was resumed whenever these passed off. There was still no sign of its suffering from diarrhœa. The respirations were 40 and the pulse 132 per minute.

July 30th.—The attendant stated that, yesterday evening, when he visited the pen, the animal was violently convulsed from time to time, but the rigid state was resumed immediately afterwards. The trismus was incessant up to the last, and was so severe that the mouth could still not be opened sufficiently to allow of the administration even of liquid nourishment.

It was found dead next morning.

The examination of the carcase was made on the afternoon of the day on which the animal died, and was to the following effect:—The legs maintained the same outstretched position as during life, and were rigid; the jaw was firmly clenched. The stomach and intestine contained a good deal of gas, so that the abdomen presented a fairly swollen appearance and was tympanitic. The peritoneal sac, however, was free from gas—at any rate, if present, it must have been in small quantity; it contained, however, $1\frac{1}{2}$ oz. of somewhat muddy-looking serous liquid. There was some amount of food in the first three stomachs, but the fourth was empty, and was free from disease. The large intestine showed a little fæcal matter, but the small intestine was devoid of contents.

There was not the slightest appearance of local reaction at the points of injection.

On the surface of the heart there were some patches of punctiform extravasation, and the blood within the chambers was softly and only partially coagulated.

The brain and spinal cord were free from visible evidence of disease; there was no meningitis.

The peritoneal liquid, on being examined microscopically, appeared to be quite free from germ impurity, the turbidity being caused by a granular precipitate.

Remarks. — This experiment was followed by very extraordinary results, and is outstanding as the only one which we have made in the inoculation of Louping-ill products where the symptoms assumed this distinctly tetanic character.

The Louping-ill bacillus was grown on 300 c.c. of glucose-beef-tea; it grew luxuriantly; the sediment of bacillus, which was copious, was mixed with sheep's blood, covered with olive oil, and incubated; the greater part of the bacillary deposit was bacteriolysed; the blood was filtered and the serum injected subcutaneously into the sheep. Symptoms did not show themselves until the twelfth day after the operation; they were ushered in with dulness, were followed by convulsive spasms of the muscles, and terminated in those of ordinary tetanus, in which last condition the animal died.

It might be argued, of course, that the case was simply one of tetanus, but against this theory is to be reckoned the fact that there was neither wound nor external injury by which the animal could have become infected, and the inoculation punctures had vanished without leaving any sign of local reaction.

Then again the sheep is not an animal which is liable to tetanus as we ordinarily understand the disease. Reference has been made, however, to the tetanus-like symptoms which occasionally develop in the course of Louping-ill, and these are sometimes accompanied by lock-jaw. The accounts we have had from shepherds and others of such anomalous cases, point to the animal becoming rigid while the jaw is firmly clenched. Such cases evidently represent the disease under conditions of the greatest severity.

The long period of incubation might be adduced as against the

symptoms having been the effect of the introduction of the serum, but then it is to be remembered that Louping-ill is a fairly chronic malady, that it sometimes runs a course of four, five, or six weeks, and that, in these cases, the nervous symptoms are best marked. We have also noticed that where the administration of the organism by the mouth has communicated the disease the symptoms have not shown themselves for a fortnight to a month afterwards. The poison of Hydrophobia requires an even longer period of incubation, and the symptoms in it, as in Louping-ill, are largely of a nervous character.

Had the animal died from tetanus we should have had to fall back on the disease being of the so-called "idiopathic" variety to account for what happened in our experiment, a disease of whose pathology nothing is known. Indeed, these examples of "Louping-ill tetanus" open up the whole question of what "idiopathic tetanus" in the human subject means.

Anæmia.

The study of this exceedingly interesting group of diseases in the sheep, and of chorea paralytica in particular, naturally leads one on to inquire whether there are any analogous diseases in the case of man. Each member of the group is caused by an anaërobic sporing bacillus whose natural habitat is the intestine, and which, under certain conditions of body, passes through the intestinal wall, and gets into the peritoneal cavity.

The anaërobic bacteria, naturally present in the human intestine, seem to include some highly pathogenic members which only require the proper surrounding conditions to enable them to call forth their toxic action. And, in the first place, certain of them have an extraordinarily powerful hæmolytic action. In the case of the group of pathogenic anaërobes parasitical on the intestine of the sheep, which we have been considering, there are also some whose hæmolytic action is intense. Among these may be mentioned Braxy and Disease "A." The organism in each of these diseases is so hæmolytic that sometimes not a single coloured blood-corpuscle will be found in the blood contained in the heart. When the organism of either of them is grown anaërobically on sheep's blood *in vitro* during the season at which these diseases prevail, the hæmolytic action is so profound that every blood-corpuscle may have

disappeared within twenty-four hours, the laking being extreme. The colouring matter of the corpuscles is not only liberated, but their stroma is dissolved, so that often not a vestige of a corpuscle can be recognised.

Curiously, the bacillus of chorea paralytica is not nearly so powerful a hæmolytic as the bacillus of either of the two diseases mentioned. The blood-corpuscles of a sheep dying of Louping-ill are preserved, and, consequently, there is an absence of blood-staining of the carcase, while, as just remarked, in the case of Braxy or Disease "A" there may not be a coloured blood-corpuscle remaining, and the carcase is deeply blood-stained.

It is a common fancy among practitioners of medicine that a large proportion of anæmias is to be accounted for by some derangement of the intestinal functions, and Hunter, as is well known, holds to the idea that pernicious anæmia is caused by an organism, probably a streptococcus, present in the intestine, and that this organism may be derived from carious teeth or patches of stomatitis.

However true this may be in general principle, it seems even likelier that the organism which hæmolyses the corpuscles is of the nature of a bacillus, anaërobic in its habit, rather than a coccus form.

I am led to this belief by the intense hæmolytic tendencies displayed by some of the anaërobic bacilli recovered from the human intestine. Thus, in the dejecta of persons suffering from progressive anæmia, I have found an anaërobic bacillus which has a most pronounced hæmolytic action. In one instance, the individual, a man, was supposed to be suffering from pernicious anæmia, so rapid and progressive was the destruction of blood-corpuscles and so characteristic were the abnormal forms. Recovery, however, ultimately took place.

The dejecta, from which the anaërobe was derived, were passed into a sterile vessel and were examined within an hour or two afterwards. From them two sets of cultures on glucose-beef-tea were started, the one aërobic, the other anaërobic. The idea was to discover whether the cultures so obtained had any effect in destroying the coloured corpuscles of human blood. The dejecta were found to be swarming with a confused mass of all kinds of organism, and at least one of them was sporing.

The aërobic growth was not purified, and presented, of course, a medley of different forms. It was desired, in the first place, to ascertain

whether these, as a whole, had any destructive influence upon the blood-corpuscles. The culture-liquid was diffusely turbid, and remained so on removal from the incubator owing to several of the forms being motile.

The anaërobic growth, on the contrary, seemed to be pure. The glucose-beef-tea on which it had been sown was heated up to 80° C. for twenty minutes, so as to destroy the non-sporing organisms. The inoculated tubes were thereafter placed in the incubator where their contents germinated with much vigour. Within five hours the contents were evolving gas, and by the end of twenty-four hours they were quite turbid. So copious was the liberated gas, that the caoutchouc caps with which the mouths of the tubes had been covered were tightly distended. Some hours after removal from the incubator, this anaërobic culture began to settle down in the shape of an obtuse cone, the apex of the cone projecting above into the clear culture liquid. It was easily disturbed, however, and the liquid again rendered universally turbid. The aërobic culture gave an alkaline reaction, the anaërobic was powerfully acid. The growth was allowed to proceed in each case for forty-eight hours.

On December 26th, 1903, an admixture of each with freshly drawn human blood was effected, in the proportion of 10 parts of blood to 1 of culture. The mixture was made in a small sterile tube with the aid of a glass rod, and thereafter aspirated into the bulb of a pipette; the ends of the pipette were sealed off and the tube placed in the incubator at 38° C. for four hours.

A control experiment was made with the same glucose-beef-tea and blood, but without organism, and treated exactly in the same fashion.

After four hours incubation the coloured blood-corpuscles in the control had fallen down to the most dependent part of the tube, while the surrounding medium was clear and unstained. The corpuscles were found to have undergone very little change. They were crenated, but yellowish-orange in colour, and in no wise exhibited signs of disintegration.

The mixture with the aërobic culture had preserved its colour fairly well, and on microscopic examination the coloured blood-corpuscles were found in a state of perfect preservation; they were not even crenated; the colourless, also, were little altered. As might be expected the organisms present were various.

The anaërobic mixture, however, presented quite a different appearance; it had only a faint yellow tint. The organism had remained in a state of purity, a rod of fairly large size, round at the ends, and apparently motionless. The hæmoglobin of the blood-corpuscles had vanished entirely, and, judging from the yellow colour of the medium, had been destroyed. The stroma of the corpuscles, however, remained, so that mere shadows of blood-corpuscles were left, with outlines so indistinct that it required careful focussing to render them visible; they were not altered in shape. The leucocytes were preserved and seemed to be quite free from any englobed bacilli.

The experiment was performed on several occasions subsequently, and always with the same result.

What the rod-organism was which had these hæmolytic properties I have not as yet determined, but certain it is that its destructive action on the hæmoglobin was most striking.

Whether it was foreign to the intestine may be questioned, indeed an anaërobic rod morphologically very like it can be isolated from the normal human dejecta, and which, apparently, has the same destructive action upon the hæmoglobin.

The anaërobic intestinal bacteria seem to be much more powerful hæmolytics than the aërobic. The majority of the anaërobes causing the above diseases of the sheep are extremely hæmolytic, and the like capacity possessed by the bacillus of tetanus, an organism evidently closely related to those of the various sheep diseases referred to, lends support to the notion of hæmolysis being a very common attribute of anaërobes as a class.

The fact that in chlorosis and anæmic diseases generally, the intestinal functions, as a rule, are in an abnormal state, evidently points the lesson that the condition of that organ is intimately bound up with the disordered state of the blood. The fact known to every practitioner that iron fails to exert its beneficial influence in chlorosis unless the intestine be got into proper working order, adds additional weight to the supposed intestinal origin of this form of anæmia in particular, and of progressive anæmias in general.

Granted that it is the poison derived from a blood-destroying organism contained in the intestine which is the cause of the hæmolysis, it may, as just said, be asked whether the organism is present normally

in the intestinal contents or introduced on occasion, whether, in fact, such a blood-destroying organism, or several of such organisms, is constantly present in the alimentary canal, and prevented from exerting its injurious influence on the blood by the interposition of the intestinal wall. Indeed it comes to be a question whether specific anæmic diseases should not be regarded as functional disorders of the intestine rather than, primarily, diseases of the blood.

We have seen that in chorea paralytica of the sheep the blood is free from organismal impurity while the intestinal contents are permeated with a highly contagious anaërobe, yet there is evidently some toxine circulating with the blood which stimulates and subsequently paralyses the motor nerve cells. The explanation I have given of the pathology of these phenomena, and the theory is founded on fairly conclusive experiment, is that a certain proportion of the bacteria which are the cause of the disease, and which are resident in the intestine, get into the blood-current and are bacteriolysed. The liberated toxins afterwards coming in contact with the nerve cells induce the characteristic phenomena of the disease.

May it not be that allied bacteria, of anaërobic habit and with hæmolysing tendencies, similarly, in certain morbid conditions of the intestine, manage to pass through the barrier constituted by its wall, become bacteriolysed in the blood-current, have their hæmolysing poisons liberated, and that such again come to act on the blood-corpuscles, either simply depriving them of their hæmoglobin as in certain of the chlorotic varieties of anæmia, or, in addition to this, effecting a solution of the whole corpuscle as in the pernicious varieties of the disease?

Tetanus.

We have seen that certain instances of chorea paralytica have quite a tetanic character, and that apparently Tetanus can be aroused in the sheep by injecting subcutaneously the bacteriolysed organism of the disease.

The organism of Tetanus has a very close relationship to that of Louping-ill, indeed to all the members of the group, and, manifestly in their all being anaërobes, in their tendency to sporulation, and when sporing, in the readiness with which they assume the drumstick con-

figuration. In the case of Tetanus, the symptoms are induced by the absorption of a toxine secreted by the bacillus of the disease. The organism exerts its evil influence only when introduced into a wound; when administered by the mouth it is apparently harmless. So universally is the organism distributed in nature that we must be constantly swallowing it, and yet no ill effects follow.

There is another variety of tetanus which is known as "idiopathic," mainly because we know nothing of its pathology, and in which there is an absence of any wound of the surface, or other apparent point of entrance. It has always been assumed that the organism inducing this idiopathic variety is the same as that occurring in the traumatic form.

In view, however, of this disease, chorea paralytica of the sheep, being so closely allied with tetanus, both with regard to the organism producing it and in the character of the symptoms occasionally evoked, I would be inclined to pause before admitting the truth of this allegation. May it not be a disease caused by an organism of the same class as that producing chorea paralytica, but intestinal in its habitat, as the organisms instrumental in producing all the members of this class of diseases of the sheep are? And may it not happen that it is a common inhabitant of the intestine, but that only in certain susceptible individuals gets over from the channel of the bowel into the blood, becomes bacteriolysed, and so allows the toxins bound up with its protoplasm to escape, these again acting on the nerve cells with which they have a combining affinity?

Tetanus of the new-born is also a form of the disease of whose pathology we have no conception, and I would go so far as to suggest that it may be the result of the action of such an intestinal anaërobe to which the infant as yet has not become immunized.

Chorea, Epilepsy, and Insanity.

The same principles applied to chorea seem to me to afford a very likely explanation of its pathology. The human disease presents many points of resemblance to chorea paralytica of the sheep, and perhaps it is not going beyond the mark to suppose that this disease of man like that of the sheep is also intestinal in its point of origin, that the same principle is at work in its production as in chorea paralytica of the sheep,

namely, that, for some reason, probably a defective condition of the blood, a particular bacterial parasite is allowed to pass over from the intestine into the blood, becomes dissolved in it, and thus has the toxins set free which are the cause of the nervous phenomena. In severe fatal instances of chorea minute abscesses have been found in the brain, and I think that quite possibly these may have been due to the organism having got into the blood in excessive quantity, so that the plasma has failed to dissolve them.

Then, again, may not Medullary Epilepsy be accounted for by a collateral pathology? May not it also be intestinal in its point of origin?

Ford Robertson (*Brit. Med. Journal*, 1901, II., p. 1230; *Ibid.*: 1903, II., p. 1065, *Edin. Med. Journal*, XIX., 1906, p. 218, Review of Neurology and Psychiatry, May and July, 1903 — Reprint), insists upon the paramount importance of the gastro-intestinal tract in relation to insanity, and makes out that in general paralysis of the insane the surface of the intestine is covered with a diphtheroid bacillus which finds its way into the tissues. He even goes the length of asserting that locomotor ataxia may be due to a similar cause.

Cirrhosis of the Liver.

It seems likely enough that Cirrhosis of the Liver may be accounted for on a pathology of the same kind. A particular bacterium becomes absorbed from the intestine, it is bacteriolysed within the portal blood, its liberated toxins are anchored upon the liver substance and stimulate this to the production of an excess of fibrous tissue. It is a remarkable fact bearing upon this theory that not infrequently the minute branches of the portal vein in cirrhosis contain organismal emboli. Quite possibly these have accumulated shortly before death, and owing to the enfeebled powers of the blood have remained undissolved.

What the ætiology of the cirrhosis of the horse due to feeding it upon lupines, or that of the cirrhosis of domestic animals said to be so prevalent in New Zealand from feeding them upon ragwort (*Senecio Jacobea*),* may be, I will not venture to broach for the present, but it seems to me these two alleged causes of hepatic cirrhosis may come to have important bearings upon the above theory.

* (See Gilruth Report, New Zealand Department of Agriculture, Division of Veterinary Science, 1902-3, p. 228; *Ibid.*, Bulletin, No. 9; *Ibid.*, Report for 1905, p. 178).

There are many other diseases which in all likelihood are conferred through the intermediation of the intestine. Thus Dr. W. Hunter, of Hong-Kong (*A Research into Epidemic and Epizootic Plague*, Hong-Kong, 1904) has been led by his researches to believe that in the case of Plague the theory of infection through the skin has been vastly exaggerated, and that a much more likely channel of contagion is the intestine. His observations are supported by Simpson's previous experiments.

Lastly, may it not be the case that a great many of the exanthemata are in reality intestinal diseases? May it not be that the intestine is the portal through which the respective poisons of these gain entrance to the system? Scarlet fever seems to me to be a case in point, for not only is there a lesion of the fauces in this disease which might quite well be a manifestation of a poison absorbed by the lymphoid tissue in that neighbourhood, but we also know that one of the commonest means of transmitting the disease is through contaminated articles of diet.

All these considerations go to demonstrate how vastly important the part played by the alimentary canal in the propagation of contagious and infectious diseases may be, and of the necessity there is for a more thorough knowledge of the whole matter. It is a field of inquiry which has been greatly neglected, unless in the case of those diseases which are manifestly intestinal in the fact of their causing some gross anatomical lesion. I would enter a plea for the more thorough investigation of those diseases whose pathology at present is unexplained, which do not leave any such residuum, and which, heretofore, have not been suspected to be of intestinal origin.

A REMARKABLE CASE OF BILHARZIOSIS.

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A Remarkable Case of Bilharziosis.

THE case that I desire to record in this volume, is one which in the extent and locality of its lesions is unique in several respects. The intestinal condition is different from anything with which I am familiar, and is the only thing of its kind that I met with during my seven years sojourn in Egypt.

The patient was an Egyptian male of about 30 years of age, who died in the Kasr-el-Aini Hospital, Cairo. The post-mortem examination was held three hours after death, on the 27th October, 1904.

The body was emaciated. Rigor mortis was present throughout. There were no external appearances of further note—no jaundice.

Weight of Organs.

Heart	-	-	-	-	175 grammes.
Right Lung	-	-	-	-	330 "
Left Lung	-	-	-	-	310 "
Liver	-	-	-	-	1225 "
Spleen	-	-	-	-	200 "
Left Kidney	-	-	-	-	120 "
Right Kidney	-	-	-	-	120 "
Brain	-	-	-	-	1205 "

THE HEART.—No pericardial fluid. The organ was small and flabby, and was dark, reddish brown in colour, being in the condition of brown atrophy. This cardiac atrophy (subject *æt.* 30) was merely a concomitant of the general advanced emaciation of the body.

THE LUNGS.—No pleural effusion, no adhesions. Both organs were small and shrunken, fairly firm and were unusually dry. The right lung was normal to the naked eye. The left lung showed on its surface a

number of small, flat, whitish nodules of firm, fibrous consistence, varying in diameter from three to five millimetres. These discs were not unlike the flat fibromata so often seen on the capsule of the spleen; they were especially seen at the margins of the organ, where they formed a discontinuous fringe resembling a row of small pearls. From analogy with bilharzial lesions, I concluded from the naked eye examination that these nodules were fibrous in nature, and would contain ova of bilharzia, but the microscope revealed quite another and unexpected condition, *viz.*, the masses were tuberculous, and were composed almost entirely of closely packed giant-cell systems, particularly at their free margin; the deeper portions (*i.e.* nearer the lung) were more fibrous in nature.

The lung itself was firm, particularly at the apex; a few small solid, pale yellow masses (tubercle) were apparent at wide intervals, and the intervening parenchyma showed under the microscope a multitude of tiny tubercles with numerous beautiful giant-cell systems, although to the naked eye this tissue appeared to be normal. The bronchial glands were but little enlarged, were jet black, and were not caseous. One of them was carefully examined microscopically, and showed neither tubercles nor deposits of ova.

There was no ascites. Numerous bilharzia worms (*Schistosomum hæmatobium*) were present in the portal vein.

The LIVER (see Fig. 1) was a typical specimen of advanced bilharzial cirrhosis, and in every particular tallied with the description given of this condition by the writer in the *Journal of Pathology and Bacteriology*, Vol. 9, p. 237.

It was slightly smaller than the average, weighing 1225 grammes, the surface presented numerous small flat fibrous growths on the capsule; the cut section showed an enormous periportal cirrhosis, "as if a number of white clay pipe-stems had been thrust at various angles through it," these white cirrhotic masses contrasting sharply with the drab colour of the general parenchyma. Microscopically, the ova of the worm were distributed in the same manner as was mentioned in my original description of this disease.

The SPLEEN and KIDNEYS showed nothing of note.

The URINARY BLADDER showed the slight roughness and peculiar sandy discolouration of the mucous membrane which is so characteristic of incipient bilharziosis of this viscus, and a scraping of these discoloured

areas showed, under the microscope, hundreds of the terminally spined ova of the *Schistosomum hæmatobium*.

The PANCREAS was to all naked-eye appearance perfectly normal, but the microscope revealed a slight amount of cirrhosis, and an unduly small number of Langherhans' islets. However, the most interesting thing noted was the presence of bilharzial ova in this organ, these ova were fairly numerous and were mostly found in the cirrhotic bands, but a few were seen lying in the glandular tissue. The ova found in the pancreas were mostly broken and partly destroyed, but the transparent broken shells were quite easily recognisable, especially as similar remnants of ova are often seen in other situations, *e.g.*, in the liver, and, moreover, it

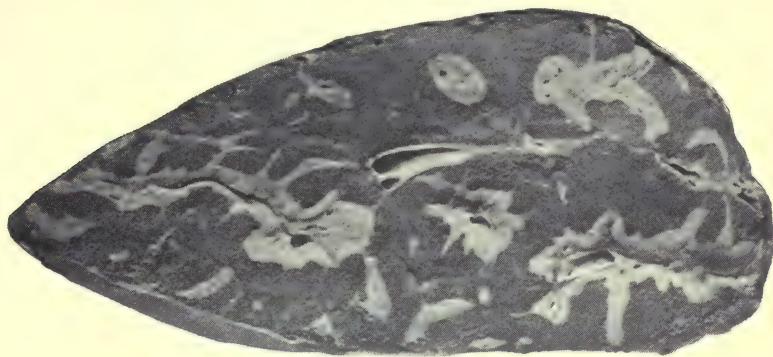


Fig. 1.—Bilharzial Cirrhosis of Liver. Antero-posterior section of right lobe. The figure shows great increase of fibrous tissue round the portal canals.

was found that certain of the broken shells retained the characteristic spine.

This is the only case in which I have deliberately examined the pancreas for bilharzial ova, and doubtless the eggs will be found in other cases, in this organ, if they are looked for. It is generally believed that the pancreas seldom or never contains these ova — probably this opinion dates from Kartulis' paper,* where the distinguished Alexandrian physician reports two cases in which he failed to find the ova in the pancreas, although they were present in various other organs. Fourteen years afterwards Kartulis again draws attention to the absence of eggs in

* Virchow's Archiv, 1885, xcix., p. 139.

the pancreas,* and quotes Lortet and Vialleton's explanation of this supposed fact, *viz.*, the female lays eggs where they may find an exit from the body of its host. Prof. Looss states that Goebel has found the ova in the pancreas.†

I venture to intercalate the remark in this place that I have twice found living worms in the pulmonary blood, one of these observations



Fig. 2.—Serous surface of ileum, showing fibrous masses of various sizes.

was published in the *Lancet* (1905, Vol. I., p. 22), the other is still unpublished. The reason the worms have not hitherto been found in

* Virchow's Archiv, 1899, clii., p. 474.

† Mense's Handbuch der Tropenkrankheiten, 1905, Vol. I., p. 96.

the lungs, is simply that they have not been looked for in a sufficient number of cases.



Fig. 2A.—Lower ileum, showing large fibrous masses on the serous surface.

The BRAIN was normal.

Before proceeding to describe the most pronounced lesion of our

present case, we may mention, for the sake of clearing the ground, that numerous specimens of *anchylostoma duodenale* were found in the small intestine; the mesenteric glands were swollen, in size from a pea to a walnut, hard and firm, and showed microscopically a marked increase of the connective tissue, wide bands of well-formed fibres, and also more delicate fibrils permeating the lymphoid tissue. Certain of

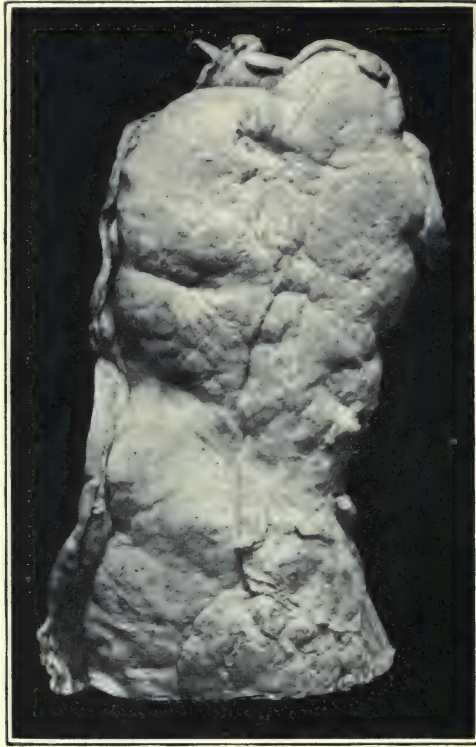


Fig. 3.—Huge fibroma on serous coat of ascending colon.

these glands contained a number of bilharzial ova, but others contained none. Here again we come upon an unusual observation — ova are not as a rule recognised as being present in the lymphatic glands; Kartulis found them, however, and mentions the fact in his paper of 1885, and again in 1899.

THE INTESTINE. — The prominent and distinctive lesion of the present case is found in connection with the bowel, particularly as regards the external or serous portion thereof.

Beginning five feet above the coecum, there were on the serous surface of the ileum a number of small polypoid outgrowths, varying in size—about a centimetre in length—consisting of a hard club-shaped extremity, often flattened or discoid, and connected with the bowel by a pedicle of loose connective tissue. These pedicles were flattened from side to side, often forming little sheets of membrane whose linear axis ran with the long axis of the gut. The distal portions of the pedicles contained the small firm masses above-mentioned, masses from the size of a split pea to a horse bean, of various shapes, discs, oryzoid, globes, round and oval masses, like a captive balloon or airship. Many of these fibrous bodies lacked the pedicle, and were connected to the gut by broad bases. As we pass down the bowel towards the coecum, the pedicles become fewer and disappear, the fibrous masses become larger, increasing in size to that of a hazel-nut, a walnut, a pigeon's egg (see Figs. 2 and 2A). Nearing the coecum the masses are several inches—four to six—in length, project an inch from the bowel surface, and occupy half or more of the circumference of the gut, looking like large sausages split longitudinally and applied to the wall of the viscus. The masses rise abruptly from the bowel, are situated on the side opposite the mesenteric attachment, and are continued almost uninterruptedly down to the coecum. They are covered by a smooth lining of peritoneum continuous with the serous coat of the bowel—show in places secondary polypoid growths, like those higher up the ileum, and are composed of dense white fibrous tissue, which contains numberless ova in certain parts.

The coecum shows a similar condition of its serous coat—a mass almost as large as a pigeon's egg. The colon is extensively affected, showing huge masses of fibrous tissue, an inch in thickness (see Fig. 3) extending along the whole length of the free surface of the bowel, and occupying from a half to three-quarters of the circumference of the intestinal tube. The secondary polypoid masses, growing from the colon tumours, were numerous and much larger than the corresponding iliac tumours, although the most extensive sessile growths were on the lower ileum. In short, as regards the external portions of the bowels, there was in the ileo-colon region a series of fibrous growths, increasing in

size from five feet above the coecum to a foot below it, thence becoming gradually smaller, to end in long thick flattened masses on the free margin of the rectum. These masses were composed of firm, well developed fibrous tissue, and contained an enormous accumulation of bilharzia ova, these latter could be seen in mass by the naked eye, being so numerous that they gave a peculiar light brown appearance to the areas where they were most abundant. Thus a cross section of one of these masses was of a glistening china white, darkened over numerous areas, to a light brown colour (see Fig. 4).

Microscopically the fibrous tissue was, for the most part, arranged in beautiful concentric whorls about the enclosed ova—an arrangement

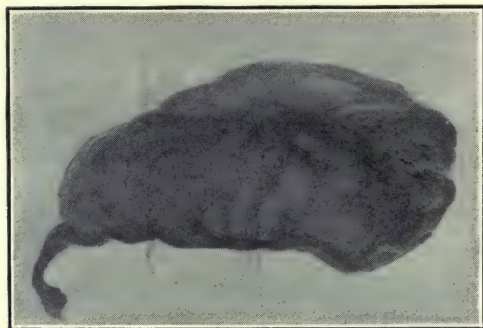


Fig. 4.—Transverse section of the fibroma represented in Fig. 3. The tag of tissue on the extreme left is a portion of the wall of the intestine, and it is seen to be continuous along the under surface of the tumour. The darker portions are enormous accumulations of ova.

which could not be detected by the naked eye. This whorled appearance was marked in the discoloured areas of the tumours, but in the china white portions (where few ova are found) the arrangement was that of ordinary fibrillar tissue.

The APPENDIX VERMIFORMIS was particularly interesting. It was distinctly thickened, measured 6 c.m. in length and 8 m.m. in diameter, being almost uniform in thickness along its whole length, though somewhat compressed to an oval about its middle portion. A mesentery was present, and opposite this attachment there was a number of delicate polypoid outgrowths from 3 to 10 m.m. in length, which had the peculiar light brown colour characteristic of an accumulation of bilharzial

ova. On transverse section of the appendix the lumen was perfectly circular, and occluded by a solid mass of soft material. External to this was a delicate whitish ring (mucous membrane), then a wide brownish ring of ova-infiltrated tissue (submucous tissue) and bounding the whole was a distinct ring of white (muscle and serosa). Thus the accumulation of ova was present between the mucosa and the muscular coat, and here the ova were innumerable, lying in a hyaline material, this region composing nearly two-thirds of the entire diameter of the appendix, the muscular coats being pushed out and partially atrophied. The increase

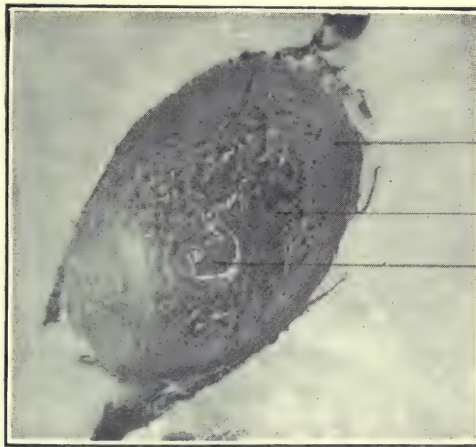


Fig. 5.—Section through appendix vermiformis (x 6).

- A. Muscular coat.
- B. Greatly thickened submucous coat, the dark areas being masses of ova.
- C. Amorphous mass in lumen of appendix.

in thickness of the appendix was due to hypertrophy of the submucous coat, and to the innumerable ova therein embedded (see Fig. 5).

Internally, the bowel was equally interesting. The mucous membrane was unduly hyperæmic throughout. Peyer's patches were normal, but the solitary follicles were somewhat enlarged. A few superficial ulcers were seen in the ileum, from one to three centimetres in linear extent, and 5 c.m. broad, they lay with their long axis traverse to the axis of the bowel, were of very slight depth, clean based with somewhat firm, rounded margins. In the ileum were a few pedunculated polypi.



Fig. 6.—Part of descending colon, showing polypoid growths. The serous portion was greatly thickened by fibrous overgrowth.

The large bowel was the seat of an extreme polyposis (see Fig. 6). Numbers of papillomata were ranged from coecum to anus, in all 120 were counted. These outgrowths varied in size from a pea to a hazel nut, some few being even larger. They were broadly pedunculated as a rule, or sessile, were nodular or mulberry-like, and were smooth or slightly roughened in places, presumably from the action of fœcal matter rubbing over them. These polypoid growths are a well-known and frequent manifestation of intestinal bilharziosis, but I do not remember

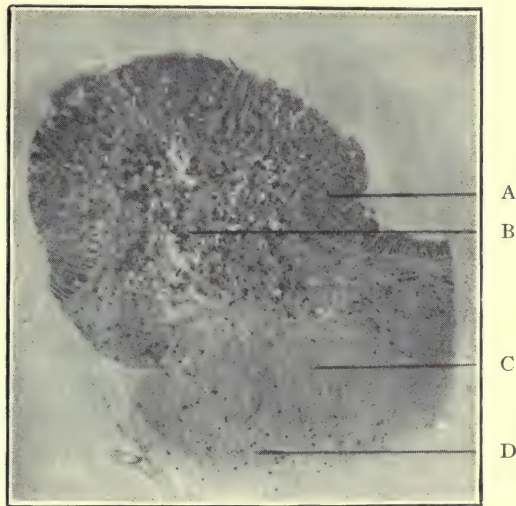


Fig. 7.—Section of Papilloma (x 6).

- A. Hypertrophied Lieberkuhnian crypts on side of tumour.
- B. Masses of ova in centre of tumour.
- C. Hypertrophied sub-mucosa of intestine.
- D. Ova in submucous tissue.

to have seen them in such numbers before, nor so uniformly scattered throughout the whole large intestine. Moreover, it is not often that bilharzial papillomata are seen above the ileo-coecal valve. I have examined a number of these growths (from the present case), and so far all that I have microscopically inspected are crowded with bilharzia ova (see Fig. 7). This is worthy of emphasis, inasmuch as the ova are very often absent from these intestinal growths.

Microscopically, these tumours show the following facts : the mucous membrane surrounding them is partially destroyed, the superficial parts thereof are worn away, the Lieberkuhnian crypts are to some extent atrophied, only their deeper-lying half is left, and these remnants show considerable alteration of the individual cells composing them, *viz.*, the luminal end of the cells is indistinct, vacuolated or empty. The crypts are separated from each other by a mass of new tissue, which, in breadth, is about equal to that of one of the crypts, and consists of cellular tissue composed of mononuclear round cells with but little protoplasm, and also of short spindle cells. A few ova are seen lying in the interglandular new tissue, but never inside a crypt itself. As this partially destroyed mucous membrane nears the tumour, it becomes greatly hypertrophied and is lifted up by the projecting papilloma, so as to form, for a short distance, a covering to the latter, but it soon is broken through by the growing tumour which is thus devoid of a mucous lining. The tumour itself arises from an over growth of the *submucous* tissue, this tissue is greatly increased in thickness along the bowel, is composed of young connective tissue interspersed by numerous groups of small round cells, and is crowded with ova. Portions of the hypertrophied submucous tissue, projects outwards through the mucous membrane, carrying with it enormous numbers of ova, and showing, in the polyp itself, a great number of congested, ectatic capillaries, and a tissue which is less highly developed than the general hypertrophied sub-mucosa, being composed of young fibroblasts, some delicate fibrils, and numerous round mononuclear cells. These mononuclear cells are in greatest abundance over the surface of the polyp, where they form a richly vascular cap of cellular tissue, which often has itself a thin covering of precipitated fibrinous material in which a few round cells are present. The papilloma contains great numbers of ova, principally in its most central part, the ova lie free in the young fibroblastic tissue, and occasionally one or two are seen lying inside a congested capillary. It is, in many instances, on the acclivities only of the papilloma that remains of Lieberkuhnian crypts are seen, though some disorganised glandular masses are found nearer the summit of the tumour. Such of the true mucous membrane as exists on the surface of the papillomata (*i.e.*, the portions spared by the faecal attrition) is greatly hypertrophied, the glandular crypts being considerably increased in length and often widened into cyst-like cavities,

besides, the membrane is traversed by numerous engorged and dilated capillaries, and thickly infiltrated with small round cells. Lying among these abnormal Lieberkuhnian crypts there are ova, but not nearly in such numbers as within the central parts of the tumours. I have found no worms in these tumours (in the present case), but the worm itself has appeared in many of the sections, lying in the sub-mucosa of the bowel, but by no means so numerous as in some cases, and moreover those I have, so far, seen are *young* males lying alone (*i.e.*, not copulated), see Fig. 8.

Hitherto I have avoided any special description of the ova themselves, because I wish to emphasise the fact that when bilharzia ova are found

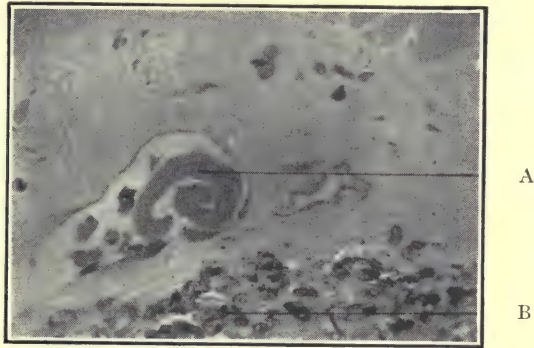


Fig. 8.—Portion of submucous tissue of large intestine ($\times 100$).

- A. Male worm in a capillary.
- B. Ova.

embedded in abnormal growths of connective tissue (or in fibro-epithelial tumours) they (the ova) are no longer living, but are dead, and for the most part disorganised. To begin with, the ova are hard bodies, and therefore cause a certain amount of tearing of the enclosing tissue when microtomic sections are prepared. Many of the ova in intestinal lesions are, as is well known, possessed of a lateral spine, now in sections of such tissues as this paper deals with, by far the greater number of the ova present no visible spine whatever, and appear completely different from normal ova such as are found in the urine. Moreover, the ova are often distorted, cracked, broken or shrivelled as if they had undergone some

solvent action, or again, empty shells, with or without lateral spines, are seen lying closely contiguous to round bodies, which are the transversely cut shells of ova which have been thus sectioned by the knife of the microtome. Never is a recognisable miracidium seen within these dead ova, but a mass of amorphous nature, deeply stainable by logwood, is all that represents the original content of the ovum. The slightly yellowish, chitinous (?) wall of the ovum is all that remains in many instances, enclosing, it may be, some dark granular material presumably calcic in nature.

To conclude, we have in the present case :—

1. A unique growth of bilharzial fibromata of the intestinal wall.
2. A marked bilharzial cirrhosis of the liver.
3. A bilharzial fibrosis of the appendix vermiformis.
4. Bilharzia ova in the pancreas.
5. Bilharzia ova in the mesenteric glands.
6. An extreme bilharzial polyposis of the large bowel.
7. A few bilharzial papillomata in the lower part of the ileum.
8. Incipient bilharziosis of the urinary bladder.
9. And, as an epiphenomenon, an acute tuberculosis of the lungs and pleura.

MALFORMATIONS OF THE BULBUS CORDIS.

An Unrecognised Division of the Human Heart.

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Malformations of the Bulbus Cordis.

An Unrecognised Division of the Human Heart.

IN this paper I propose to set out as briefly and clearly as I may, the evidence which has led me to the two following conclusions:—(1) that besides the sinus venosus, the auricular and the ventricular divisions—the three parts out of which the whole mammalian heart is believed to be formed, there is present a fourth, namely, the bulbus cordis; (2) that the great majority of cases which are classified, at the present time, as congenital stenosis of the pulmonary or of the aortic orifices are, in reality, due to an arrest of development or malformation of the bulbus cordis. It is with the second of these two conclusions that I now intend to deal here, and it will be apparent that if the congenital constrictions, which are found at the orifices of the pulmonary artery and aorta, can be shown to be due to an arrest or vitiation of development, then the theory, which has still a wide currency amongst medical men that these lesions are the result of fœtal endocarditis, must be finally abandoned, and these conditions classified with hare-lip, cleft palate, hydrocephalus, spina bifida, club-foot, atresia ani, hypospadias, and other congenital malformations with which the cardiac lesions are so often associated.

NOTE.—The interpretation which I give of the malformations of the heart described in this article is a new one, and it is not necessary, therefore, to give an exhaustive list of references. As regards our present knowledge of this subject, Peacock's work *On Malformations of the Human Heart* (2nd Edition, London, 1866), is still the most authoritative work which has been produced in England; Rokitsansky's *Die Defecte der Scheidewände des Herzens* (Wien, 1875), holds a corresponding place in German medical literature, while Moussous' *Maladies Congénitales du Cœur* (Paris, 1896), and Théremin's *Etudes sur les Affections Congénitales du Cœur* (St. Petersburg, 1895), may be accepted as representative of the more recent work done in France and Russia.

This article deals with one of the results of an effort I have made during the last two years to systematise our knowledge of congenital malformations of the heart. I have had an opportunity of examining over 200 malformed hearts in the medical museums in London (50 of these were obtained at the London Hospital). In the Transactions of the Pathological Society of London, I obtained descriptions of 123 cases; from other sources in medical literature I obtained over 200 cases more, so that, altogether, I have accounts of over 500 specimens. The Index Catalogue, issued from the Surgeon General's office of the United States, contains 12 columns of references to records of isolated cases of congenital *morbus cordis*, and it was an examination of some of these records which convinced me of the necessity of a systematisation of our knowledge of this subject.

To understand the nature, structure and primary connections of the bulbus cordis, one must study this chamber of the heart in fishes, of the shark or allied tribes, in which it reaches its highest structural and functional development. It is the last of the four pulsating chambers of the primitive vertebrate heart. In figure 1, these four chambers are shown, the sinus venosus (1), a reservoir into which the great veins

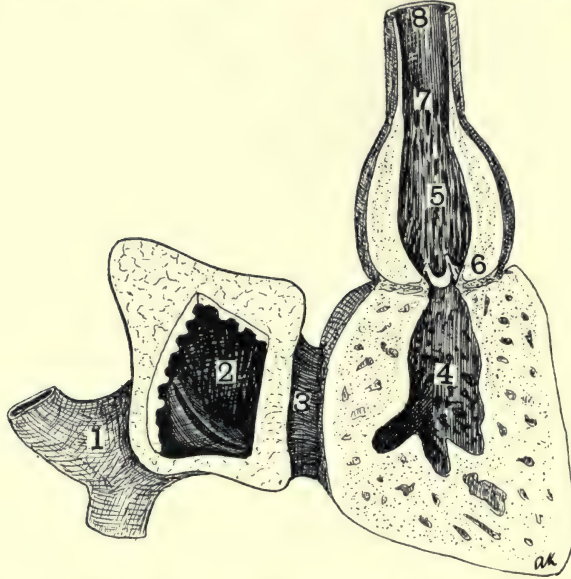


Figure 1, heart of *Xiphias gladius*, showing the four primary compartments of the heart (from a specimen in the Museum of Royal College of Surgeons, England).

- (1) Sinus venosus.
- (2) Auricle laid open, showing the venous valves guarding the orifice of the sinus.
- (3) Auricular canal.
- (4) Ventricle.
- (5) Bulbus cordis.
- (6) Lower orifice of bulbus (ventricular orifice).
- (7) Upper " (aortic orifice).
- (8) Common ventral aorta leading to gills.

discharge their blood, the common auricle (2), the essential function of which appears to be that of loading the ventricle; the common ventricle (4), and the bulbus cordis (5), out of which opens the primitive ventral aorta leading to the bronchial circulation. It is a matter of regret that there is so little direct experimental evidence of the exact part played by

the bulbus cordis in the circulation of fishes, but from an examination of its structure, we may refer, to some extent at least, its functional significance. A lower orifice (figure 1, 6), usually provided with valves, marks the separation of bulbus and ventricle; an upper orifice, which may or may not be valvular, indicates the junction of bulbus and primitive aorta. The walls are usually thick, and composed of a dense musculature; its cavity, fusiform in shape, with a corrugated, ridged or valvular lining of endocardium. Its musculature is of the striped cardiac type, rhythmically contractile and inhibited by the action of the vagus (*Gaskell*).

The bulbus cordis is commonly regarded as a sphincter mechanism to secure competency of the aortic valves. The necessity for this elaborate mechanism becomes more evident when it is remembered that the entire heart of a fish is purely a branchial or respiratory pump, and discharges its load, not into the whole arterial system, as in the higher vertebrates, but into the limited capacity of the branchial system, which must undergo a relatively great expansion with each heart beat—its valves being so rendered incompetent. The valves are made competent by virtue of the musculature which surrounds them, therefore it is possible that the bulbus, by the degree of its tonus, may also regulate the blood pressure within the branchial system, either by permitting a functional regurgitation or regulating the rate of outflow from the ventricle into the aorta. Such a theory of the function of the bulbus does not explain the form and great development of the bulbus, nor the great thickness and rhythmical contractions of its muscular walls. It may be provided with valves only at its upper or at its lower orifice. In fishes, in which there is a well developed bony operculum covering the gills, the bulbus is small or vestigial. The movements of the operculum probably regulates and assists the circulation of blood through the gills. Further, there is this most important fact to bear in mind that with the evolution of a pulmonary system and its complete separation from the systemic circulation the bulbus cordis becomes restricted to the right side of the heart, and can act therefore only on the pulmonary circulation. Whatever future research may show, this much is certain that the bulbus cordis is essentially a part of the respiratory system, and apparently is chiefly concerned in regulating the flow of blood through the lungs. In this connection it seems pertinent to recall the fact that physiologists have failed to demonstrate a vaso-motor supply to the pulmonary vessels.

Any descriptive account of the anatomy of the heart, even by those who write clearly and well, is particularly hard to follow, hence it is necessary for me to use every aid to make my description intelligible. This end will be served if, at the outset, I indicate by the use of a figure, the part of the normal human heart which, on the evidence to be produced, I believe to be formed from the bulbus cordis. That part is the infundibulum of the right ventricle (see Fig. 2). The wall of the right ventricle, when opened in the manner shown in figure 2, is seen to show

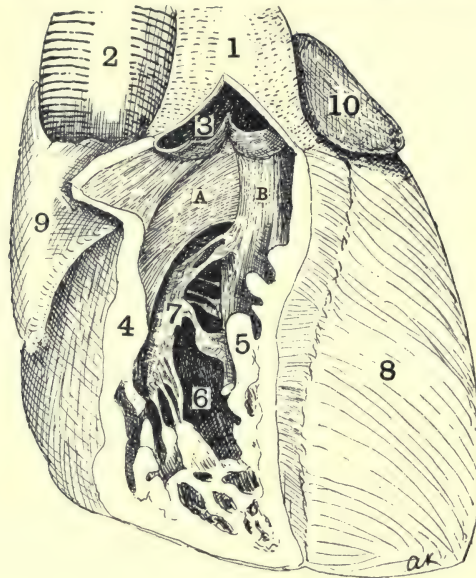


Figure 2, normal human heart with the right ventricle opened.

- (1) Pulmonary artery.
- (2) Aorta.
- (3) Pulmonary valves (upper limit of infundibulum).
- (4) (5) Thickenings in wall of right ventricle at the junction of the infundibulum and body of the right ventricle.
- (6) Body of the right ventricle.
- (7) Tricuspid valve.
- (8) Left ventricle.
- (9) Right auricle.
- (10) Left auricle.

A, right septal band, B, left septal band of infundibulum. The line between A and B indicates the position of the septal raphe of the infundibulum.

a distinct thickening at the points indicated by 4 and 5. These thickenings mark the junction of the infundibulum with the body of the right ventricle, and represent the position of the lower or ventricular orifice of the bulbus cordis. On the septal wall of the infundibulum are seen two muscular bands or plates, the right (A) and left (B) septal bands, with between them a faintly marked and rather diffuse raphe. The septal wall of the infundibulum, formed by the two muscular bands or plates just mentioned, is only 20 to 25 *mm.* in length, while the lateral or marginal wall of the infundibulum is twice that depth or more. Its muscular walls are dense, the fibres are in the main circular in direction; the uppermost fibres surround the base of the pulmonary valves, and, as is well known, render these valves competent by their tonus in diastole. The infundibulum of the right ventricle is manifestly different in structure, and presumably also in function, from the body of the right ventricle, and I regard it as derived directly from the bulbus cordis, the muscular thickening between the two parts of the ventricle representing the ventricular or lower orifice and the pulmonary opening the aortic or upper orifice of the bulbus cordis.

In studying the evolution of the mammalian from the primitive four-chambered heart of the fish, one recognises that three great changes have taken place; (1) the primitive auricle and ventricle have become completely divided into right and left chambers; (2) the sinus venosus has become partly or, as in man, almost completely, submerged in the musculature of the right auricle; (3) the bulbus cordis has become separated from the left ventricle and aorta, and completely incorporated in the right ventricle as the infundibulum of that chamber. That such was the fate of the bulbus cordis in the mammalian heart, I became convinced four years ago from a study of the malformations and comparative anatomy of the heart, but my evidence was incomplete until Greil published the results of an exact and complete research into the embryological history of the bulbus cordis, and demonstrated its fate in the mammalian heart. (For abstract of Greil's research see Hochstetter's account in *Handbuch der Vergleich. u. Experim. Entwicklungslehre der Wirbeltiere*, parts 4, 5, 14, 15, 1903). The cavity of the bulbus cordis is incorporated in the right ventricle by an upgrowth of the ventricular musculature round it, the musculature of the bulbus being thus replaced by the musculature of the ventricle, in the same way as

the musculature of the auricle replaces a great part of that of the sinus venosus. The submergence of the bulbus evidently constitutes a critical phase in the developmental metamorphosis of the heart, and it is during the critical phase that malformations are apt to occur and give rise to the various lesions I shall now proceed to describe.

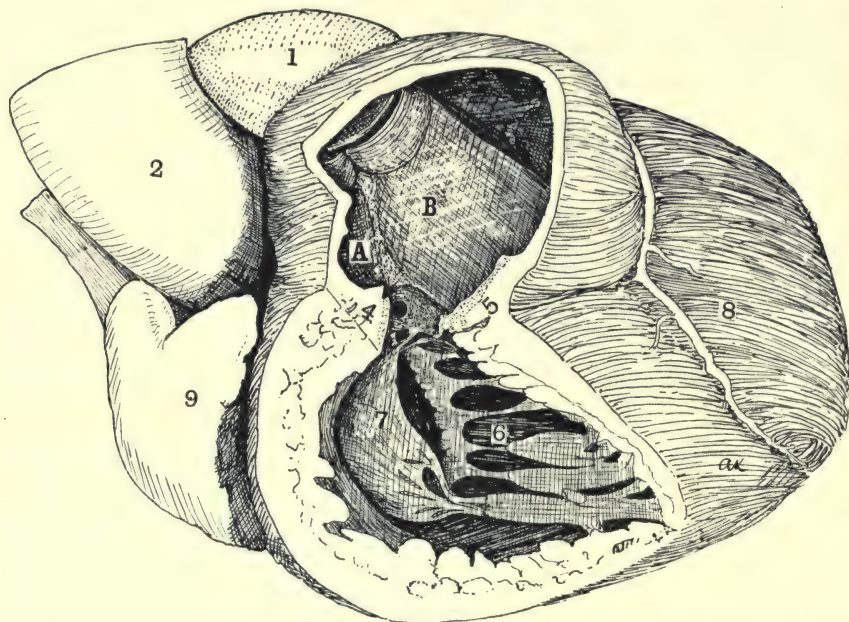


Figure 3, heart of a man aged 20 who died, after two months' illness, from an acute infection with endocarditis.

- (1) Pulmonary artery.
- (2) Aorta.
- (4) (5) Fibro-muscular constriction marking the lower infundibular or bulbar orifice.
- (6) Body of right ventricle.
- (7) Tricuspid valve.
- (8) Left ventricle.
- (9) Right auricle.
- (A) Right septal band.
- (B) Left septal band.

Between A and B is seen a wide infundibular raphe.

Between 4 and 5 is seen a small interventricular foramen.

The first class of cases I wish to deal with is that described as division of the right ventricle; they are really cases in which the

infundibulum and body of the right ventricle have developed to a normal extent but they have never completely fused, a constriction remaining between them, representing the ventricular orifice of the bulbus. A typical specimen is shown in figure 3. It is the heart of a young man who sought treatment at the London Hospital, and was for two months under the care of Dr. Percy Kidd, to whom and to Dr. F. J. Smith I am indebted for the opportunity of examining this specimen. The infundibulum is enormously dilated as may be seen from the figure, and is sharply separated from the body of the right ventricle by a muscular partition, which is perforated by an orifice, 10 *mm.* in diameter. The orifice is surrounded by dense fibrous tissue which resembles, in structure, the tricuspid valve. Just below the orifice is seen a small interventricular foramen (see figure 3, between 4, 5). The infundibular raphe (between A and B), ends at the bulbar orifice; the septal bands A and B are wide and well marked. The fibrous tissue round the constriction I regard as a representation of the valves and fibrous tissue at the lower orifice of the bulbus cordis (see figure 1). This condition is certainly not common, but in the same week as I obtained the specimen figured here, I received another from Dr. John Hay of Liverpool. It was the heart of a woman who had reached middle age; the infundibulum was smaller and its orifice not so narrow, as in the specimen figured here. Altogether, I have had an opportunity of examining 15 specimens of this type, and about 50 cases are recorded. In some of them the constriction between the infundibulum and body of the ventricle is but slight, and the fibrous tissue of the margin is represented by a ring of irregular endocardial elevations. The explanation which has hitherto been offered of such a condition is that it is the result of cicatrisation following an endocardial lesion. Evidence in support of such a hypothesis is entirely lacking.

The second class of cases which I now proceed to deal with are common, and are usually described as congenital stenosis of the pulmonary artery; they are, in reality, due to an arrest of development of the bulbus cordis. In the first class of cases, just described, there was no arrest of development of the infundibulum—the condition was due simply to a persistence of the lower bulbar orifice. In this group, however, the condition is different (see figure 4); usually the bulbus is only partly expanded; the endocardial lining is thick and frequently cor-

rugated, like the bulbus of fishes ; the lower orifice may be marked, as in the case shown, by a fibrous diaphragm, muscular in structure at its circumference. The muscular walls are extremely thick, as if the proper amount of muscle had been provided for a fully expanded infundibulum. The pulmonary orifice may be constricted ; the valves may be free but are commonly imperfectly separated. A wide interventricular orifice is commonly present, and death usually occurs before twenty. Death is

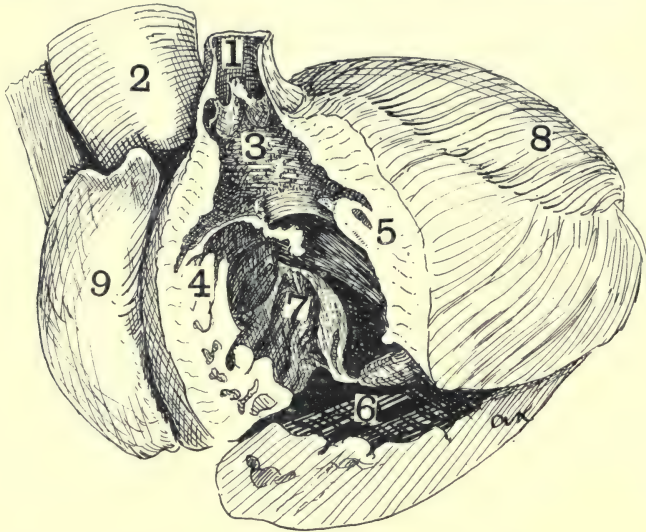


Figure 4, heart of a girl aged 7 years. The right ventricle has been opened by a triradiate incision.

- (1) Pulmonary artery (narrow, thin-walled).
- (2) Aorta (large).
- (3) Infundibulum below adherent pulmonary valves.
- (4) (5) Orifice of infundibulum ; below orifice is a large interventricular foramen.
- (6) Body of right ventricle.
- (7) Tricuspid valve.
- (8) Left ventricle.
- (9) Right auricle.

frequently caused by an attack of bronchitis or of pneumonia, and in the majority of cases recent vegetations are found round the fibrous margin of the orifice. It is the occurrence of these vegetations which has induced so many clinicians to accept endocarditis as the cause of the entire condition ; it is evident that no endocardial lesion could produce

this form of infundibulum; the right interpretation appears to be that these congenital constrictions are specially susceptible of becoming the site at which vegetations form.

In the third class of cases of malformation of the bulbus cordis, the arrest of development is more complete than in the second class, but of course stages intermediate to these two classes occur. The condition is shown in figure 5, where the heart of a boy aged 6 yrs. with the right ventricle laid widely open is represented. The infundibulum is small

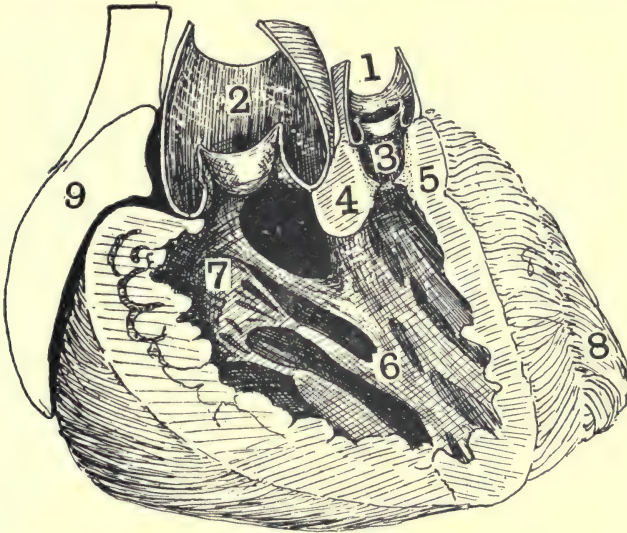


Figure 5, heart of a boy aged 6 with the right ventricle laid widely open.

- (1) Pulmonary artery.
- (2) Aorta also laid open; below its orifice is seen a wide interventricular foramen leading into the left ventricle.
- (3) Infundibulum or bulbus arrested in its expansion.
- (4) (5) Lower orifice of infundibulum or bulbus. 4 represents septal band A in section (see figures 2, 3, 4).
- (6) Body of right ventricle.
- (7) Tricuspid valve.
- (8) Left ventricle.
- (9) Right auricle.

(7 mm. in depth, 4 mm. in width), lined by opaque thickened endocardium, with a well-marked narrow lower orifice, formed by a fibrous thickening

of the endocardium. The upper or pulmonary orifice is guarded by two valves partially adherent. The orifice is so narrow that very little blood could have entered the pulmonary artery from the right ventricle. The aorta opens directly from the right ventricle, and the septal bands

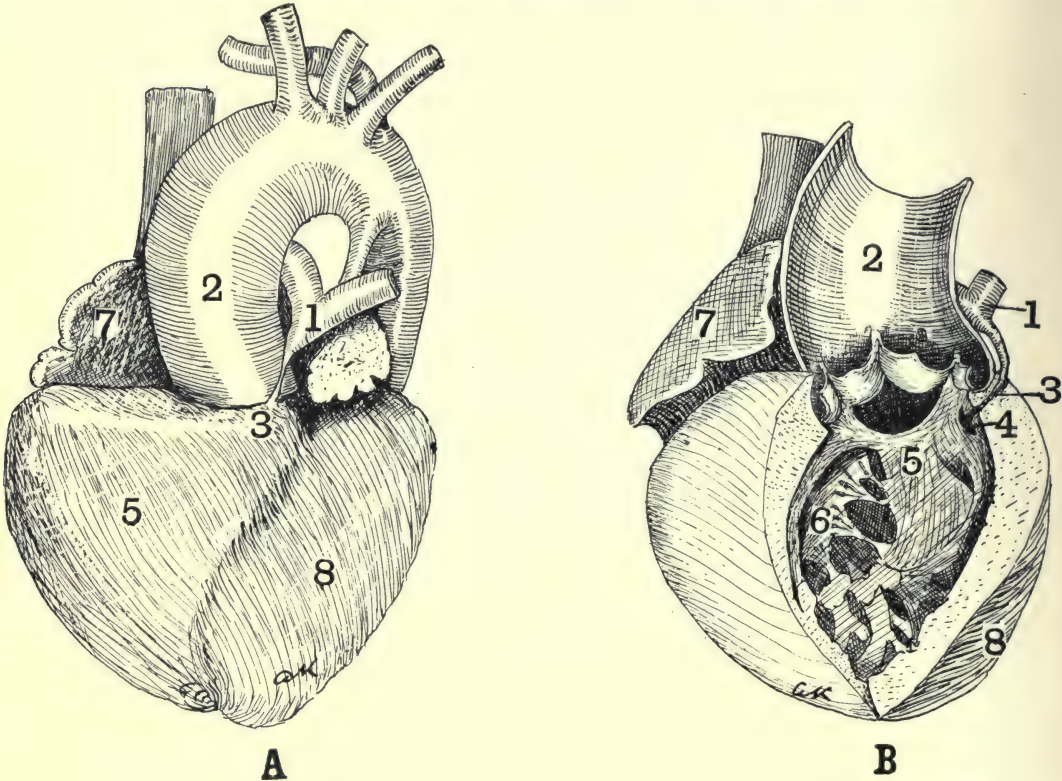


Figure 6, heart of a girl aged two years. A represents the heart unopened; B the right ventricle laid open. (The specimen was given to me by Dr. J. A. Milne.)

- (1) Pulmonary artery.
- (2) Aorta.
- (3) Position of infundibulum or bulbus.
- (4) Lower orifice of infundibulum or bulbus.
- (5) Body of right ventricle. (Above 5 in figure B is seen the interventricular foramen.)
- (6) Tricuspid valve.
- (7) Right auricle.
- (8) Left ventricle.
- (9) Left auricle.

marked A and B in figures 2, 3, form here functional parts of the wall of the right ventricle, which throws its contents into the aorta. This class of case and the one described just before this, make up over 50 per cent. of the cases usually classed under congenital pulmonary stenosis.

In the cases dealt with so far, the bulbus has been arrested in its growth and expansion after the upgrowth of the ventricular musculature, but there still remains for consideration a very considerable group of cases, in which the arrest of development is almost complete. In figure 6 a case of this kind is represented; in that figure two views are given of the heart of a girl aged two years. In A, the heart is represented unopened; the commencement of the pulmonary artery was represented by a thread of tissue; in B the right ventricle is opened, and all that is seen of the infundibulum or bulbus is a slight cleft with fibrous lining exposed by section of the wall of the ventricle, just in front of the aortic orifice. The septal bands A and B of the infundibulum are shown completely fused in the muscular wall of the ventricle, and unless one knew where to look and what to look for, the remaining trace of the bulbus would pass unobserved. The various conditions of the infundibulum of the right ventricle, which I have just described, constitute by far the great majority (over 90 per cent.) of the cases classed as congenital pulmonary stenosis. It will be seen that the condition is usually one which affects much more than the orifice of the pulmonary artery; it involves a complete segment of the right ventricle. Further, the condition is not one which can be accounted for by a foetal endocarditis; the form and extent of the lesion can only be accounted for by accepting the hypothesis that the infundibulum is derived from the bulbus cordis, a fusiform chamber, with a thick, endocardial lining, which becomes incorporated in the right ventricle during the first month of development, and whatever agent may be at work in producing this malformation must exert its effect at this early stage of development.

Having now indicated the part of the right ventricle, which is derived from the bulbus cordis, and the numerous malformations to which it is liable, it is necessary to explain the manner in which the bulbus cordis becomes restricted to the right side of the heart. When it is remembered that the aorta, as well as the pulmonary artery is derived directly from the primitive aortic stem, it is apparent that, in the mammalian heart, both of these vessels ought to spring from the part of the heart

which represents the bulbus cordis. The aortic part of the bulbus atrophies and disappears, and in order to understand this alteration, I propose now to describe briefly the metamorphic changes which, thanks to the labours of His, are known to occur in the human heart during the third and fourth weeks of development. The condition of the human heart during the fourth week is shown in figure 7; A represents the condition about the beginning of that week, and B a stage towards the end of the same week. Both are taken from illustrations given by His.

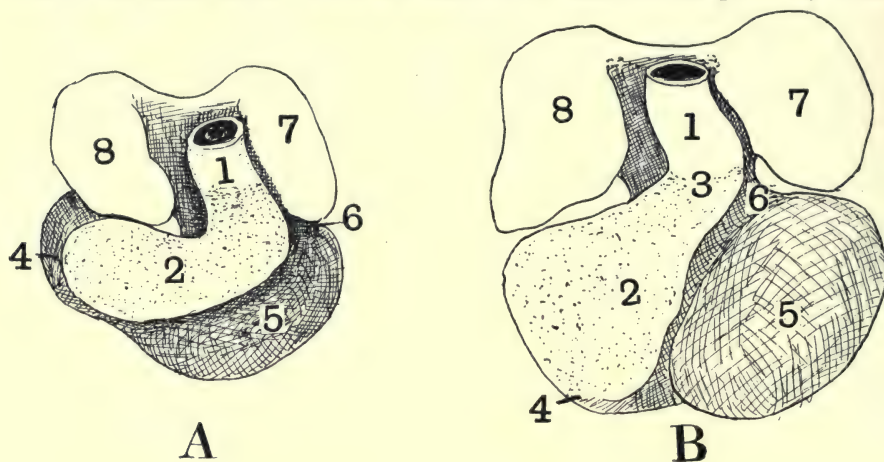


Figure 7, A, heart of a human embryo, 4·5 *mm.* long. (His).

B, heart of a " " 5 " long. (His).

- (1) Primitive aortic stem from which pulmonary artery and aorta will be developed.
- (2) Bulbus cordis.
- (3) Upper or aortic orifice of bulbus cordis.
- (4) Lower or ventricular orifice of bulbus cordis.
- (5) Ventricle.
- (6) Auricular canal (auriculo-ventricular junction).
- (7) Left auricle.
- (8) Right auricle.

To assist in the interpretation of these hearts, I give a figure of a fish's heart (*Echinorhinus spinosus*) drawn from the same point of view—in each case the sternal aspect of the heart is represented. In their shape the ventricle and bulbus of the fish's heart recalls the stomach; the auricular canal (6) corresponds to the oesophagus; the auriculo-ventricular junction, the oesophageal orifice, the ventricle, the cardiac part of the

(68)

stomach ; the bulbus, the pyloric part of the stomach ; the upper orifice of the bulbus, the pyloric orifice. Lesser and greater curvatures are distinctly marked, the lesser curvature being the result of a restricted growth along one margin of the cardiac tube, which brings the upper orifice of the bulbus towards and against the auriculo-ventricular orifice, in the same way as an exaggeration of the contracture of the lesser

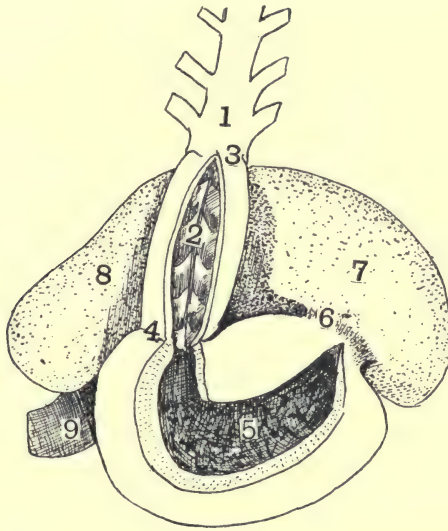


Figure 8, heart of a fish (*Echinorhinus spinosus*) from a specimen in the Museum of the Royal College of Surgeons, England.

- (1) Primitive aortic stem giving off branchial aortic arches.
- (2) Bulbus cordis.
- (3) Upper orifice of bulbus.
- (4) Lower orifice of bulbus.
- (5) Ventricle.
- (6) Auricular canal.
- (7) Left auricle.
- (8) Right auricle.
- (9) Sinus venosus.

curvature of the stomach would approximate the pyloric and oesophageal orifices. In the fourth week, the parts of the human heart correspond in shape and position to those of the fish just described (see figure 7, A, B), there being this difference that the lesser curvature is undergoing a rapid

atrophy, with the result that the lesser curvature of the bulbus is closely applied to the lesser curvature of the ventricle, and the upper orifice of the bulbus is being brought directly in front of, and applied to, the auricular canal (see figure 7, A, B). The lesser curvature becomes rapidly absorbed.

The changes which result from the complete atrophy of the lesser curvature will be best appreciated by an examination of two diagram-

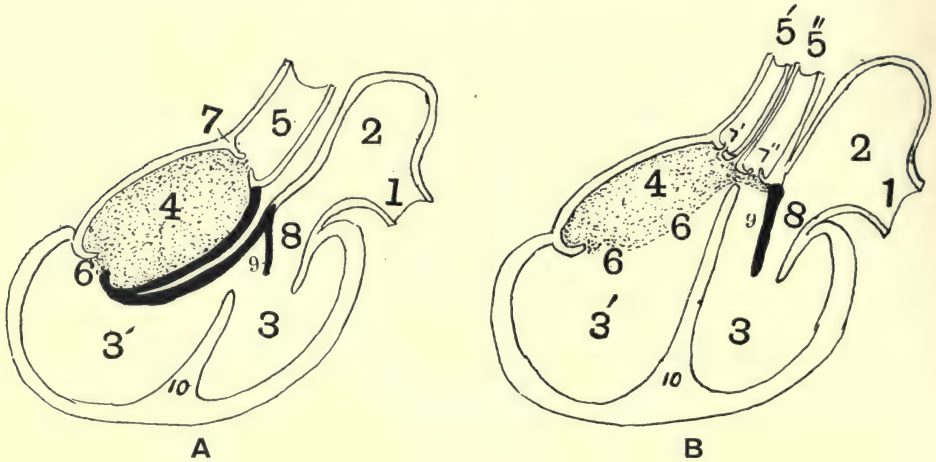


Figure 9, (A) Diagrammatic section of the embryonic heart showing the lesser curvature by a black line.

(B) The same showing the result of an atrophy of the lesser curvature.

- | | |
|----------------------------|--|
| (1) Sinus venosus. | (6) Lower orifice of bulbus. |
| (2) Auricle. | (7) Upper " |
| (3) Ventricle (left). | (7') Orifice of pulmonary artery. |
| (3') Ventricle (right). | (7'') " aorta. |
| (4) Bulbus cordis. | (8) Auriculo-ventricular orifice. |
| (5) Primitive aortic stem. | (9) The anterior cusp of the mitral valve. |
| (5') Pulmonary artery. | (10) The interventricular septum. |
| (5'') Aorta | |

matic sections of the embryonic mammalian heart which are reproduced in figure 9. In each section the four chambers of the primitive heart are shown; (1) sinus venosus; (2) auricle; (3) ventricle; (4) bulbus. In A the lesser curvature is represented by a thick black line. The atrophy of the lesser curvature is seen to result in the following

modifications :—(1) The adjacent sides of the bulbus and ventricle fuse and rapidly disappear, and thus the bulbus comes to open freely into the right ventricle (see figure 9, A, B); (2) the lesser curvature is represented by only the base of the mitral valve (see fig. 9, B, 9); (3) the lower or ventricular orifice of the bulbus is carried up to the base of the anterior cusp of the mitral valve; (4) the upper or aortic orifice of the bulbus becomes applied to the base of the anterior cusp of the mitral. Further, it is to be noted that the aorta arises from the part of the bulbus which undergoes atrophy in the disappearance of the lesser curvature of the heart (see figure 9, B, 7"). It is in this manner that the part of the bulbus corresponding to the origin of the aorta becomes stretched and disappears, while the part of the bulbus which remains, representing almost the entire bulbus, becomes incorporated in the right ventricle.

Thus it comes about that the part of the bulbus cordis corresponding to the origin of the aorta disappears completely at a very early date, and usually not a trace of it is to be seen at the aortic opening of the left ventricle. Occasionally remnants do occur; all the cases showing such a condition which I have had an opportunity of examining have been regarded as malformed by the cicatricial effect of endocarditis, but a closer examination of the condition, and a fuller understanding of the development of the heart, show that such an interpretation is untenable. In figure 10 is given a representation of the heart of a man, aged 20, who died recently in the London Hospital. It shows a condition which I interpret as being due to the persistence of the bulbus cordis round the orifice of the aorta from the left ventricle. From figure 9, B, it will be seen that such a remnant should form an endocardial ring round the aortic orifice (the area derived from the bulbus is stippled in figure 9, B). The case is one in which there was an acute infection of both mitral and aortic valves, with ulceration and formation of vegetations. But the stout fibrous ring which surrounds the orifice of the aorta, below the aortic cusps, and is continuous with the fibrous tissue of the heart from which the aorta arises, could not be produced by any lesion which is known to occur in acute or chronic endocarditis. On the mitral valve, the ring is represented by endocardial elevations which, in section, are seen to be made up of, not inflammatory tissue, but of one closely resembling Whartonian jelly, a tissue in which branched cells are embedded in a jelly-like ground

substance. The fibrous ring round the rest of the orifice has the same structure as the mitral valve—fibrous tissue with the fibres lying in parallel bundles, with cells arranged in definite lines between the fibres.

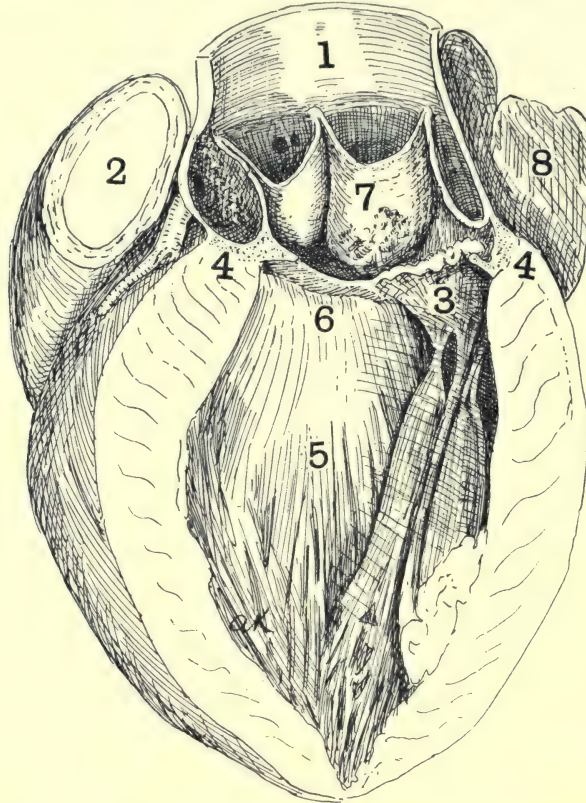


Figure 10, heart of a man, aged 20, who died in the London Hospital from acute endocarditis. The left ventricle is opened and exposed.

- (1) Aorta.
- (2) Infundibulum of right ventricle.
- (3) Anterior cusp of mitral valve.
- (4) Base of left ventricle; between 4, 4, below the origin of the aorta is seen the fibrous endocardial ring, which I interpret as a remnant of the bulbus cordis.
- (5) Left ventricle.
- (6) Top of interventricular septum, below fibrous ring.
- (7) Mitral cusp of aorta, showing ulceration and vegetations.
- (8) Left auricle.

Further, I have been able to collect nine cases exactly similar to this, but the interpretation of the cause of the condition is rendered difficult by the fact that in every case endocarditis was present. The ostium bulbi on the right side of the heart, when it persists, is specially liable to become the site of an endocardial lesion; it seems to me that this explanation may be also employed for the left side of the heart; a constriction such as is caused by a persistent part of the bulbus cordis is especially liable to become the site of endocarditis. Further, the constriction which is seen in this and in similar cases, occurs at the position and in the form which one would expect from a persistent remnant of a bulbus cordis on the left side of the heart.

It would carry this paper beyond its proper compass were I to describe cases in which the bulbus cordis has atrophied round the pulmonary artery, and not only persisted but attained a great muscular development round the origin of the aorta; in this form of maldevelopment is to be found the explanation of the not uncommon condition of transposition in the origin of the aorta and pulmonary artery. Nor do I intend to deal with that form of atrophy of the aortic part of the bulbus which leads to complete aortic stenosis, so common in Russia and so rare in England. I have gone far enough to show that the theory of foetal endocarditis cannot account for the various forms of congenital lesion which I have shown to occur in the heart, and the causation of which has to be sought for in the first month of foetal development. Further, these malformations cannot be accounted for except on the hypothesis that the bulbus cordis forms an intrinsic part of the mammalian heart; the bulbus is not completely absent in the mammalian heart, as has been the current opinion up to the present. That it does exist in the form described we have as evidence not only its persistence as the result of maldevelopment, but also the facts of embryology and comparative anatomy.

To render the evidence more complete, that these lesions are not the result of foetal endocarditis, but of some agent, the nature of which we do not yet know, I propose to give in this paragraph the results of the examination of the heart in a group of 23 malformed human foetuses and infants. The malformations which these foetuses and infants showed were one or more of the following: anencephaly, hydrocephaly, spina bifida, umbilical hernia, atresia ani, cleft palate, hare-lip and stenosis

of the oesophagus. Of these 23 cases, 14 showed a malformation of the heart; of the 14, 10 showed lesions of the bulbus cordis; in some cases it was the pulmonary part of the bulbus which was non-developed, in others it was the aortic. The important point is that these lesions were similar to those I have just described in older hearts. It appears from this evidence, then, that the malformations of the bulbus are the result of the same agent as produces the condition of anencephaly, etc., with which lesions malformation of the bulbus is so often associated.

THE ADMINISTRATIVE ASPECTS OF
TUBERCULOSIS.

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The Administrative Aspects of Tuberculosis.

I. The Tubercle Bacillus—Tuberculine—Heredity.

How does the problem of tuberculosis present itself to the administrative mind? To that question I shall try to give some answer.

For the modern administrator, the history of tuberculosis began when Koch isolated his bacillus. That the disease was an infection, communicable from man to man, is a fact as old at least as the days of Isocrates, and older. Through the ages, the belief in its infectivity can be traced in literary and scientific records. The Nineteenth Century cannot claim to have discovered the fact, nor can the Twentieth Century yet claim to have exhausted the pathology of the disorder. But it remains true that, for the ends of administration, the whole history of the disease before Koch may be blotted out of our books. Even with the isolation of the bacillus, the administrative problem was weighted by a thousand irrelevancies. The pre-Koch pathology is far from dead. It still perverts the clinical mind. It is still repeated in the text-books. It still crowds the lectures with antiquarian rubbish. It clouds the mind of the student with useless knowledge. It blocks the way to frankness of outlook and precision of practice. Curiously, it has faded most rapidly where the lay mind has had to be convinced. For, to teach the farmer, or the salesman, or the butcher, or the dairyman, or the mother of children, or any of the other innumerable units that constitute an organized society, all the delicacies contained in the ancient theories of a wasting disease would have been a hopeless and futile task. Even the youngest medical officer of health—fresh, enthusiastic, full of Virchow and not ignorant of Darwin—would have been beating his head against the rocks had he tried to rouse in the lay mind any interest answering to his own. Until Koch, the disease was too difficult, too complex, too

little understood, to be taught to any but technically-trained people. But when Koch came, a note of hope rang round the world. He passed through the fire of criticism, not scathless, but carrying with him his cardinal fact—that where his bacillus was, there also was tuberculosis. The word tuberculosis passed from the vagueness of speculative pathology into the circle of positive science. It was henceforth to mean something as definite as gunpowder, or oxygen, or steam. Forthwith, tuberculosis became a doctrine that the lay mind could grasp. It could be taught as easily as the multiplication table, and it could be shown to be as practical.

So far, well. But this alone, though it excited the hopes of the world and simplified the duty of the administrator, would not have secured the growing interest of the layman. To him a new germ may be an interesting curiosity; he will listen to tales about it; he will take pride in repeating its name. But he is nothing if not practical. If you cannot do him some definite good, you will tire his interest and you will provoke him to reaction. Fast on the heels of the new bacillus came the suggestion that the disease due to it was no longer hopeless and incurable. Then the whole world began to ask for a miracle. It seemed for a time as if the miracle had happened and the diseased were to be made whole. The heart sank when the signs failed. But the miracle had indeed happened, although the revelation of it was looked for too soon. Science on the one hand, and, on the other hand, Nature came, once more, together, and the open-air treatment became a fact. Meanwhile, science pushed forward more and more intensively until new facts, new methods, new habits of the organism revealed themselves, and now, after all, the tuberculine cure of tuberculosis is no longer a dream of possibilities, but a definitely established doctrine. The conditions are not so simple as the natural feelings led us to imagine; but they are not so complex as to have baffled the patience of research. The day is here when, not as a vague belief resting on unsolved mysteries, but as a permissible deduction from ascertained fact, the forecast of the near future may be—tuberculosis will be extirpated.

So far, again, well. But in the popular mind there was another obstacle. Biology, on the authority of great names, had left us with a crude theory of inheritance. What could it profit that we isolated the bacillus if the personal pedigree were bad? Did we not hear tales of

families swept away, member after member, each when his day came? Were we not filled with horror at the inherited taint? Did not the insurance companies, do they not still, base their calculations on the belief that a phthisical inheritance ought to mean a loaded premium? And in some sense, they are perhaps justified. But the countenance began to lighten and the action to grow athletic when it was, again after long research, made clear that tuberculosis is not inherited—that it is mainly a thing of the environment. It is, in fact, a struggle between two organisms, a lower and a higher. The lower is the invader, the parasite; but the higher has now become the master. The vague hopes of the earlier days are now planted firmly on a basis of definite inductions. The bacillus can be isolated; it can be killed; it can be traced into a thousand by-ways; it can be stopped at a thousand points of its path from one mouth to another; it does not pass from generation to generation.

To the administrator, the isolation of the bacillus made the problem simple. To the reformer, the growing belief in the non-inheritance of the disease has offered a new basis of action. The reformer is justified in taking as his objective a possible society without tuberculosis. The administrator has now to devise the methods of attaining that end.

II. Tubercular Death-roll for Scotland.

How does it stand with Scotland? Here I have no case to prove; I seek only to indicate what can be done. But a single figure may be taken as a starting point. For the present period, some six thousand persons die in Scotland every year of pulmonary phthisis; some four thousand die of other forms of tuberculosis. These figures are probably less than the truth; for good as our methods of diagnosis are, multitudes of cases still escape scientific scrutiny. Probably, five times ten thousand gives some approximation to the number of tubercular persons *living* in Scotland. In this, however, our data are still insufficient. But for our present purpose one solid fact is enough—ten thousand persons die of tubercular diseases, and a vast number will soon follow them. The administrative problem is how to delay for the longest possible time the death of the infected, how to reduce the number of possible infections, how most rapidly to convert a widespread plague into an occasional outbreak.

III. Death-rates and Deterioration of Stock.

In passing, perhaps it is worth noting that to reduce the death-rate of a disease does not mean to confer immortality on the patient. If he does not die of phthisis, he will die of something else; but it is probable that if he does not die of phthisis, he will live at least a little longer before he dies of anything else. So elementary a fact should not be worth stating; but it is worth stating, for I seem to detect in the occasional disappointment with the slow fall of death-rates an unexpressed, perhaps unconscious belief, that they ought to go down to nothing per cent., which is immortality. This, of course, is not compatible with the conditions of life known in this world; we must be satisfied with deferred death. The important fact is that when we save a case from death by consumption, we are effecting a real, not an apparent, saving. For there is no evidence that the extirpation of consumption means the establishing of any other fatal disease. It has been contended that by saving a larger number of tubercular people—of course the tubercular diathesis is itself an hypothesis only—we ultimately contribute to the deterioration of the race by a geometrical increase of feeble constitutions. The argument applies to every infection; but by long tradition it has, in the popular mind, a special application to phthisis. Personally, I have not been able to discover a sound reason for the argument. If we make it cut the other way, we ought, logically, to assume that every cause of death whatsoever permits the survival of a greater number of deteriorated stocks. But it is possible to classify causes of death into selective and non-selective. There is, however, no obvious or immediate test to enable us to discriminate which disease is selective and which non-selective. It is pure hypothesis to assume that phthisis is one of the selective causes, and that every person that dies from it is better dead than living. Neither for phthisis nor for any other infection have we any data of the slightest value to enable us to give a conclusive solution of that problem. In our efforts to limit the spread of tuberculosis, we may assume, meanwhile, that every reduction in the death-rate is a social gain. It is pitiful to learn that superstitions about Natural Selection, which is not a god, but an abstraction, sometimes divert public funds to other uses. It is more pitiful to learn that men with an elementary training

in science are capable of offering those perfunctory half-thoughts as if they were established truths. Before we determine dogmatically who shall die, we should exhaust our means of discovering who can live.

IV. General Sanitation versus Direct Control.

Ever since Koch's discovery, conviction has been gaining ground that the spread of tuberculosis can be limited by administrative methods. In many parts of Europe and America, it has been so limited. There is a good deal of evidence for the proposition that the isolation of phthisical cases has materially reduced the total number of cases. It is true that, for fifty years, the death-rate from phthisis in Britain has fallen year by year until to-day it is only about 50 per cent. of what it was. It has been assumed, without much effort at analysis, that this steady decrease has been the result of improved "general sanitation." Doubtless, general sanitation has contributed something, if under "general sanitation" we include the draining of soils, the sewerage of towns, the improvement in houses, the increase in cleanliness of habit, and most of all the steady, remorseless, systematic campaign against infection of every form. What destroys one infection destroys another. Incidentally, in our efforts to limit typhus, typhoid, puerperal fever, scarlet fever, diphtheria, septicæmia, pyæmia, and many other infections, we have been dealing, intimately and in detail, with the same conditions as the tubercle bacillus thrives in. In killing typhus and typhoid, we have, doubtless, without intending it, killed also tuberculosis, but the tubercle bacillus is a slowly invading and most persistent parasite. It gets to places that few other parasites can invade. Everywhere it finds a nidus so easily that it is naturally the last to be expelled. Precisely because it kills slowly, it kills most. That is probably why, when most of the other parasites steadily fall back before isolation, disinfection and prophylactic injections, phthisis needs more determined and subtle dealing. But with every allowance for the insidiousness of this slow parasite, we are now justified by the evidence in our conclusion that by direct attack, as in typhus, typhoid, scarlet fever and the others, we shall be able to reduce the spread of the disease by securing that the patient shall confine his infection to himself. To

this we are now able to add direct methods of cure. When cure and prevention can, as they ultimately will, work perfectly together, tuberculosis will fall back to the social status of plague and cholera in the western world.

How this end may be attained, the pioneer agencies in every land have already shown us. The curative side has been made manifest by the rapid growth of the Sanatorium. The preventive side has yet to receive its full share of attention. Here I propose to indicate how, with our present legal resources, we may, in Scotland, turn our official battalions against Pulmonary Phthisis, which is, for practical purposes, the only grossly infective variety of tuberculosis.

Let it be assumed that there are three, roughly separated, classes of cases—incipient cases, intermediate cases, and advanced cases. Let it also be assumed that each case runs an irregular history, varying from weeks to years. Let it still further be assumed that thousands of patients, though affected in varying degrees with the disease, are yet fit to pursue their callings and to maintain economic independence for years. Here we have all the factors of the administrative problem.

To meet these what have we administratively? We have sanatoria; general hospitals; infectious disease hospitals; poorhouse hospitals; methods of disinfection; educational organizations; housing improvement schemes; improved drainage, water and ventilation; inspection of factories, of workshops and of work-places; vast legal powers for the removal of nuisances, the prevention of overcrowding, improvement of house structure, selection of house sites, cleansing of houses, cleansing of persons, the isolation of patients, the supervision of dairies and the milk supply, the examination of cows, the destruction of tubercular meat, the maintenance of paupers and their dependents, dispensaries, and many other minor powers and organizations. To see that the laws are effectively applied for all these objects we have the local authorities for public health, that is the town councils in towns and the district committees in counties; we have the parish councils, who must deal with phthisis as one of the largest pauper-producing agencies. In the sequel, I shall show how these official and unofficial agencies may be brought into perfect correlation and so secure that one vast organised army shall march forward to the extirpation of the enemy.

V. What are the Organisations Available?

The available organisations may be classified into Public and Private. Public organisations are the Local Authorities for Public Health in Town and County; the Parish Councils; the Charitable Hospitals with their outdoor departments; the charitable Dispensaries. The Private organisations are:—the special Homes, Hospitals and Sanatoria, devoted exclusively, or almost exclusively, to the treatment of phthisis. The campaign against phthisis ought, as Dr. R. W. Philip has held for many years, to correlate all the organisations Public and Private.

In Scotland, the Local Authorities for Public Health number some 313. Of these, the majority are Town Councils; the minority are District Committees. Of the Town Councils many are small, they have control only of small resources, and their efficacy for any extended Public Health movement is correspondingly small. But in a large number of cases they are incorporated with the District Committees, which are the Local Authorities for Public Health in the counties. On the other hand, the death-rate from Pulmonary Phthisis is usually highest in the towns, and the burden on the Town Councils is, therefore, greater. But the phthisis death-rate also stands high in the counties, and in some counties it is much higher than in others. In the large cities, the phthisis death-rate is highest, and in them, fortunately, the resources are greatest of all. Obviously, the practical policy is for the smaller towns to co-operate with the county districts. Above all it is necessary for both orders of Local Authority—Town Authority and District Authority—to avoid the mistake of attempting, out of their own resources alone, to solve a problem that more than any other requires combination and concerted action.

The Parish Councils number over 900. Their primary business is to administer the Poor Law. They must take charge of the indoor and outdoor poor. They must organise indoor and outdoor relief. They must have Poor Inspectors; they must have Poorhouses; they must have Medical Officers of Poorhouses; and they must have Medical Officers for the outdoor poor. In Scotland, there are some 69 Poorhouses. It is practically no direct part of the Parish Council's duty to prevent infectious disease; their duty is to prevent and to control

pauperism. But among the causes of pauperism one of the chief is Pulmonary Phthisis. And, as it is the duty of the Parish Council to take medical charge of the sick, they must incidentally treat phthisis. This they cannot do without proceeding, to some extent, on the modern lines both of prevention and of treatment, and on those lines they have largely proceeded. For several years the Local Government Board for Scotland have, through their Superintendent of Poorhouses, systematically year by year inquired into the methods employed in the Poorhouses for the isolation and treatment of phthisis and other forms of tuberculosis. The result has been that, in the majority of the 69 Poorhouses, segregation of phthisical cases is more or less completely carried out. In several, the open-air methods have been organised. The cases isolated are usually chronic, advanced and dying cases, which, as a result of the relative segregation established by residence in the Poorhouse, have, in greater or less degree, been removed from the general social circulation. As time goes on, the numbers so segregated will certainly increase. Every patient segregated even for a time is at least one focus of infection temporarily extinguished. But again as time goes on the Public Health relations of the disease will become more manifest, and the action of the Local Authority for Public Health will become more imperative.

Of the other public institutions, such as hospitals and dispensaries, many make some effort towards treatment, and a few are reserved exclusively for phthisis. Among the pioneers in this path is Dr. R. W. Philip, Edinburgh, who, nearly twenty years ago, established the Royal Victoria Dispensary for Tuberculosis and the Royal Victoria Hospital at Craigleith. The details hereafter given of the proper functions of a municipal or county dispensary are based on the results achieved by Dr. Philip, who has time and again pressed upon the public the necessity for official organisation and correlation of agencies. His fundamental idea, as given in detail below, is that the Municipal Dispensary should be, as it were, the Court of First Instance for every case, selecting those suitable for Sanatorium or for palliative treatment, or for complete isolation; investigating the homes, controlling the industries, distributing information, and, in general, serving as a directive bureau and exchange for the record and correlation of all preventive and curative agencies.

It is obvious that the charitable organisations shade into the private

organisations, and are capable of being worked in association with them. Here it is unnecessary to give further detail.

What, then, can the Local Authorities for Public Health do? It is on them that the chief burden of the campaign must now fall; for they have the powers and the duty to deal with the disease.

VI. Disinfection.

The Public Health (Scotland) Act contains many clauses that deal with disinfection. These are all available for Pulmonary Phthisis as for any other infective disease. Probably they were drawn up primarily for the suppression and prevention of the common infections like scarlet fever, diphtheria, enteric fever, typhus fever, smallpox, etc. But, when carefully examined, they are found applicable even to a peculiarly long and variable disease like phthisis. The Local Authority can, and, when required by the Local Government Board, must provide disinfecting appliances and the officers necessary to work them (46).^{*} They may disinfect or destroy, as necessity indicates, bedding, clothing and other articles that have been exposed to infection. These they may remove and destroy or disinfect and return free of charge. For all these purposes, they may combine with other Local Authorities.

The Local Authority may also cleanse and disinfect premises when it is certified by a Medical Officer of Health or a qualified medical practitioner that such cleansing and disinfection would tend to prevent the spread of infectious disease (47). They may require householders or others concerned to carry out the disinfection, or they may through their own officers and at the public expense themselves disinfect the premises, etc. To those familiar with the persistence of the tubercle bacillus, it hardly needs to be said that, to be effective, disinfection must be very thorough-going. Recently the perfunctory methods common in the last generation, such as fumigation with sulphur, have steadily receded before the more effective application of disinfectants by washing, moist brushing, or spraying. For clothing, the methods now employed are steeping in disinfectants of adequate strength, or passing through saturated steam under pressure. In some places, for the disinfection of

^{*}The numbers refer to the sections of the Public Health (Scotland) Act, 1897.

rooms infected by phthisis, the favourite method is washing the walls with freshly made solution of bleaching powder (strength 1 per cent.). In others the method is spraying of walls, floors, ceilings, furniture, etc., with sufficiently strong solutions of formaldehyde, or carbolic acid, or corrosive sublimate, or one of the many effective disinfectants now available. In a room infected with the tubercle bacillus, it is absolutely essential that all the dust should be sterilized; otherwise the room cannot be regarded as safe. And it is not enough that the objects in the immediate vicinity of the patient should be dealt with; it is equally essential that the floors, walls, furniture, etc., should be treated with the same scrupulous detail as if the room were to be prepared for an aseptic surgical operation. But if this is to be done, obviously it requires skilled and experienced officers to do it.

Further, when a patient is treated at home, the clothing and the room should be periodically disinfected. Works or workrooms where phthisical patients are employed should be treated in the same way. In no case should they be left to the chance and perfunctory methods of the unskilled and uninformed.

Are we not asking too much? Is it necessary, in practice, to lay such stress on infected rooms and infected workshops? The evidence leaves us no alternative but to say that it is absolutely necessary. Case after case has been known to result from sleeping in infected rooms and infected bedding. Of the cumulative proof towards establishing the practical infectivity of phthisis, no part is more striking than the clinical and experimental evidences gathered from infected sick-rooms.

Other Sections of the Act (51 and 53) confer special powers for dealing with infected houses, or infected houses re-let without disinfection.

Other Sections (50 and 56) enable the Local Authority to disinfect public places and control the public movements of infectious persons. For instance, no person suffering from any infectious disease ought wilfully to expose himself without proper precautions against spreading the disease in any street, public place, shop, inn, hotel, church, or any place used in common by persons other than members of the family or household to which the infected person belongs. And, as by Section 50, it is an offence to expose infected matter in ashpits, etc., clearly the Local Authority may, if they choose, prevent phthisical persons from scattering their infected sputum in public places. The practice of

uncontrolled spitting doubtless is a factor in perpetuating the circulation of the tubercle bacillus. Spitting ought everywhere to be controlled. Schools, churches, halls, theatres, markets, railway stations, railway carriages, tramcars, omnibuses, hackney carriages, etc., etc., ought to be periodically cleansed and disinfected.

The Local Authority should also make use of their powers under Section 58, and see that no person suffering from Pulmonary Phthisis, or living in a house infected with that disease, shall milk any animal, or pick fruit, or engage in any occupation connected with food, or carry on any trade or business in such a manner as to be likely to spread the disease. The powers of this Section are of primary importance.

VII. Isolation and Treatment.

NOTE.—The remainder of this article, except the concluding paragraphs, is taken from a circular issued by the Local Government Board for Scotland in March, 1906.

The provisions of the Public Health Act as to removal of cases of infectious disease to Hospital (54) and as to the provision of hospitals and houses of reception (66) are available for dealing with cases of phthisis as with cases of other infectious diseases. These provisions are comprehensive and elastic, and can be adapted in practice to any type of case—incipient cases where the danger of infection to others though, for the time, at a minimum, may suddenly become serious; intermediate cases, where the patients, still able to work, may, if uncontrolled, become dangerous; and advanced cases, where the patients, frequently unable to attend to themselves, may be a source of grave danger.

Hospitals for Pulmonary Phthisis may be classified as follows :—

A. *Curative Hospitals (Sanatoria) for early cases.*

Here the object is, by generous and selected food, by life in the open air, and by other forms of recuperative treatment, to develop the resistance of the body to tuberculosis, to produce a cure of the affected lungs, and to restore the patient to normal efficiency. To achieve these objects, it is necessary to have the patient closely supervised and carefully nursed. The conditions of sanatorium life are in many respects the reverse of those in the homes whence the patients come. The patients themselves require courage and perseverance. This treatment has been more

successful than any other hitherto attempted. It is now being supplemented in some sanatoria by other forms of special treatment. The progress of the patient towards increased constitutional resistance can be tested from day to day and week to week by methods of extreme delicacy. Continuous and stringent medical supervision is essential.

It is obvious that, to produce satisfactory results, such highly specialised Hospitals must be in the hands of specialists experienced and skilled in the methods of scientific diagnosis and treatment. Otherwise, patients and their friends will be disappointed. Accordingly, individual Local Authorities, instead of attempting themselves alone to provide the trained medical skill and scientific laboratories necessary for effective management, should either utilise existing sanatoria or combine with other authorities for a properly equipped sanatorium. It should be part of the duty of the Local Authority, or Phthisis Committee, to discover and to keep in touch with all available sanatoria, either in the immediate locality or at a distance.

It may be added that, in structure, sanatoria may be of the simplest and cheapest form. Wood and iron sanatoria have been provided at Woodilee and Gartloch Asylums at approximately £90 per bed. Open-air shelters can be provided at a cost of from £6 to £8 per bed, or even less.

B. All-Day Hospitals.

In some sanatoria, patients attend all day for treatment, and return home at night. This method has been found very satisfactory where the patient's home is reasonably suitable. The resources of Hospital treatment can thus be made available without any serious increase of ward accommodation. Extra shelters are alone necessary. The patients are not only educated in open-air methods; they are definitely treated with a view to complete cure. Doubtless, as the curative methods are further improved, day-attendance at Hospitals will grow in favour. The patients have the advantage of being nursed and medically supervised through the greater part of the day.

C. All-Night Hospitals.

Frequently, the patient's home is unsuitable for living in over-night. But his work may be in the open air, and in every respect compatible

with the treatment of the disease. In such cases, arrangements might be made for his going to work during the day and sleeping in the Hospital at night. He would thus be kept under continuous medical supervision, and at the same time enabled to follow his occupation without danger to others. This arrangement could be made at a Sanatorium or other Hospital or Colony.

D. Convalescent Colonies and Homes—Work Colonies.

It has been found that patients, after treatment in a Sanatorium, are apt to relapse when they return home. Accordingly, it is desirable to provide Convalescent Work Colonies or Homes for such cases. Such Colonies or Homes, if well organised, might be made almost, if not entirely, self-supporting. The patients would be specially selected. They would continue the open-air treatment. They would be provided with light labour, as a preparation for their ultimate return to full work. Certain satisfactory experiments have been made in this direction.

E. Hospital Wards for Educative Treatment and Control.

In several localities, vacant wards of the Hospital for ordinary infectious diseases have been used for educative treatment of phthisical patients. The most striking example is the town of Brighton. Here, patients are admitted for one month. They are placed under full Sanatorium treatment for the time. They are taught to sleep with wide-open windows or in the outdoor shelters. They are taught how to disinfect and dispose of their sputum. They are provided with sputum flasks and paper handkerchiefs. They have their blood examined as a test of their condition. Meanwhile, their homes, their clothing and bed-clothing are disinfected. Their friends are instructed in the open-air methods and in the precautions necessary for safety. Their employers are communicated with and advised. At the end of the month in Hospital, they return home. They are then visited periodically by officers of the Medical Officer's Staff. They are further assisted when necessary. In the event of death, disinfection is thoroughly carried out.

In this way, large numbers of cases are brought under systematic control. The cost to the Local Authority of Brighton is relatively small. This system, which was first elaborated by Dr. Arthur Newsholme,

Medical Officer of Health, is capable of being organised, on some scale, at trifling expense, wherever there is a vacant Hospital Ward and an adequate medical nursing and public health service.

F. Hospital Wards for Isolation of Advanced Cases.

Many patients are too advanced in the disease to leave their beds. Their homes may be entirely unsuitable for safe nursing. Such cases should be isolated. For this purpose, vacant wards of an Infectious Disease Hospital may be utilised. Here, palliative treatment is possible. There will be adequate medical supervision and nursing. Under very simple precautions, friends and relatives may have access to the patient.

In many localities, notably in Lanarkshire, much has been done in this direction. The isolation of such dangerous cases is a primary duty of the Local Authority. By Professor Koch and many other investigators, these advanced cases are regarded as the principal sources of infection.

It will thus be seen that every variety of case may be suitably dealt with by the Local Authority.

VIII. Dispensaries for Pulmonary Phthisis.

"In towns and other thickly populated localities, where the number of phthisical patients is large, the Local Authority will find it advisable to institute a Dispensary or Dispensaries. In Edinburgh, the Royal Victoria Dispensary for Tuberculosis, organised by Dr. R. W. Philip, has worked successfully for eighteen years, and the suggestions here made are largely based on the experience of that Dispensary. (See paper by Dr. R. W. Philip, *Edin. Med. Journal*, January, 1906.) On the Continent, notably in France and Germany, Dispensaries have been of immense value in the discovery of insanitary conditions, and in the organisation of nursing service.

The work of a Phthisis Dispensary would include the following :—

- A. Medical Examination of Patients—either at the Dispensary or in their own homes.
- B. Inquiry by a medical man, or qualified nurse, into the history of the illness, the home-conditions, the economic condition of the family, the occupation, the suitability or unsuitability of the accommodation for home-treatment, &c.

- C. Arrangements for providing medical treatment and nursing of patients that could be treated at home without risk of infection.
- D. Dispensing of medicines, and disinfectants.
- E. Arranging for periodic washing and disinfection of clothing when the conditions render this necessary.
- F. Selection of cases suitable for any of the forms of hospital treatment enumerated above. This is one of the most important parts of Dispensary work.
- G. Distribution of printed information among patients and their friends.
- H. Arrangements for examination of sputum and other discharges.
- I. Arranging for the removal of cases to hospital.
- J. General medical supervision of cases not removed to hospital, and general guidance in all matters concerning tuberculosis and its control.

The Municipal or District Phthisis Dispensary ought to be the Central Bureau of information. It should keep a register of all sanatoria, hospitals, infirmaries, work-colonies, convalescent homes, parochial hospitals, private houses, phthisis committees, and all other organisations that, either within the district or without, can be made available for the inhabitants."

IX. Notification of Pulmonary Phthisis.

"For the effective application of the Public Health Act to Pulmonary Phthisis, a system of Notification is essential. In some localities, a system of Voluntary Notification has been organised.

"But it is open to the Local Authority, with the approval of the Board, to add Pulmonary Phthisis to the list of diseases notifiable under the Infectious Disease (Notification) Act, 1889. The Board will be prepared to give favourable consideration to any application for their approval provided they are satisfied that the Local Authority are in a position and are ready to deal effectively with the cases notified to them. Notification of itself has no administrative value, and unless it is to be followed by effective measures for curative treatment of the patients and for prevention of the spread of infection the Board will not

feel justified in approving of the compulsory notification of cases of the disease."

Long ago, when laymen and very few medical men were agitating for the notification of the ordinary infections, the same arguments were heard: the "liberty of the subject" always "blocked the way." To-day, the subject's liberty is secured by the very Acts that seemed certain to destroy it. The apparent coercion of the Notification Act of 1889, and its precursors, was only the civil instrument for unveiling to the citizen mind a new social duty, namely, the duty to save his fellow and, therefore, himself, from the dangers of preventable infection. In the Statute-book there is no Act that works with greater smoothness or greater effect. And its application to Pulmonary Phthisis is only a matter of detail. The principle is everywhere accepted. But Notification is only one step. It is right to take it. It will be taken. But it must be prepared for, it must be followed, by effective action in every case. Meanwhile, cases are waiting in thousands to be received, and, for the moment, compulsory notification is a secondary point of tactics. In Scotland, compulsory notification of the common infections did not become general until 1897. It does not yet, except in one or two places, apply to whooping-cough, or measles, or diarrhoea. But when methods of controlling those diseases are revealed, notification will be swiftly invoked. In Phthisis, methods of control are obvious and effective, and notification will soon be asked for everywhere as a simple necessity of administration.

X. Organisation and Administration.

"It has been shown above that the Public Health Act applies to Pulmonary Phthisis; that, for a successful campaign against the disease, the Local Authority must employ the various institutions and agencies suited to the various types of patients; that sanatoria, hospitals, and dispensaries must be worked always in concert, and that a system of notification, voluntary or compulsory, is indispensable. It remains to add that an organisation so highly specialised demands the services of a special Sub-Committee of the Public Health Committee.

This Sub-Committee—the Phthisis Committee—should have charge of the Dispensary, which, as indicated above, should be the Administrative Centre of the whole Organisation for dealing with Phthisis. The

Dispensary will normally be under the direct management of the Medical Officer of Health, and will constitute a special department of his work.

The organisation of hospital service—sanatorium and other—would naturally be part of the duties of a Phthisis Committee ; but the details will vary according to the institutions available in or for the District."

XI. General Sanitation.

"It should be unnecessary to remind Local Authorities that these direct measures towards the control of Pulmonary Phthisis must be supplemented by indirect measures, the unremitting and systematic removal of nuisances, prevention of overcrowding, enforcement of good ventilation, reconstruction of insanitary houses, improvement of insanitary areas, improvement of drainage of soil and houses, stringent supervision of meat, of cowsheds, of dairies, cleansing of streets, proper disposal of refuse, &c. Direct prevention should go hand in hand with general sanitation."

XII. Conclusion.

It is often said that the law as it stands is not adapted to the administrative control of Pulmonary Phthisis. It is alleged that new powers are necessary ; that the present powers may bear too heavily on individuals or particular classes ; that the peculiar economic relations of phthisis may involve such interference as would disturb the existing organisation of society to its foundations. It is suggested that if the clauses of our Public Health Act were enforced for phthisis as for infections like enteric fever or small-pox, the lives of thousands would be made intolerable ; that, in the great industries, workmen would boycott their infected or infective fellow-workmen, and that the hardship, the misery, the poverty necessarily resulting would be a greater evil than the persistence of the disease or its slow evanescence. These fears date from a time when the knowledge of infection and its habits was rather a superstitious belief than a reasoned doctrine. They claim to be founded in a sensitive care for the economic welfare of the working classes ; they are more reasonably assigned to an uninformed imagination. At the very moment when such fears are the commonplace of discussion, tens, hundreds, thousands of cases are clamouring at the doors, thinking little

of "social ostracism," thinking a great deal of the treatment they need and can secure only at the hands of the public organisations. Indeed, the fear of social hardship is rapidly vanishing before the verifiable facts of the open-air treatment and the growing evidence of anti-toxic cures. The powers of our Acts are now, when cases are being handled by the thousand, found to be after all nothing but the simple necessities of the administrative problem. The Acts are strong, but the clauses are elastic. The highly developed Public Health Service of Scotland has, for fifteen years in the counties and for a longer period in the large towns, been finding its way into the most intimate recesses of social life. What ten years ago presented itself to the lay mind as a "medical fad" is now the commonplace of every town council and every district committee. The demand for isolation, for disinfection, for hospital treatment, comes no longer from the medical side; it comes imperatively from the side of the patient. By the systematic administrative handling of the other infections, the public mind has been educated to accept a similar treatment of phthisis. The time has now come when the executive authorities, who have many of them been doing a little, may, with safety and the certainty of universal assent, make a great concerted movement to limit the spread of the disease.

No longer is it necessary to deal with private organisations alone, with charitable societies, infirmaries, or dispensaries. It is possible, it is necessary, that we should put in motion the statutory organisations at present charged with the control of infectious diseases. True, it is easier to agitate for new legal powers than to execute in detail the powers we have. It is easier to stir opinion than to superintend spade-work. The one requires only an interest in the subject; the other demands systematic action day by day. But as the case stands, the general conviction of the medical profession has at last found a response in the general conviction of the lay mind. The public authorities are ready. The vastness of the work to be done has somewhat appalled those unaccustomed to handle so great a movement. But the necessity for handling it has become irresistible. And, after all, when the campaign is thought out, when the organisations available are contemplated, the problem, in passing into detail, passes easily into practice. We cannot at one stroke undo the work of centuries, but in a single generation we can make an effective beginning.

PAROXYSMAL IRREGULARITY OF THE HEART
AND AURICULAR FIBRILLATION.

BY

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Paroxysmal Irregularity of the Heart and Auricular Fibrillation.

IN December, 1901, our attention was drawn by Professor Peterson, Director of the gynecological clinic in the University of Michigan, to a case of marked irregularity of the heart occurring in his wards. Careful examination of the heart was made, and a large number of sphygmographic tracings were obtained. Unfortunately, we were not acquainted at the time with Mackenzie's methods of taking the venous and liver pulses, and failed to take advantage of this most valuable method of analysing cardiac irregularity. The case is of itself of considerable interest however, and the light which seems to be thrown on it by our experience of irregularity in animal experiments, encourages us to put it on record.

The history of the case supplied by Dr. Peterson is as follows :—

Mrs. H. H., widow, aged 64. Admitted to hospital, December 23rd, 1901, for ovarian fibroid ; operation December 24th. Anæsthesia (ether) for 1 hour and 20 mins. Condition good at end of operation, pulse 90. Nausea persisted throughout the 25th and 26th, the patient vomiting repeatedly, but on the 27th some improvement was noted in this respect. The pulse on the 25th and 26th was 90-100 at the visiting hours, but the nurse stated subsequently that she had noticed frequent irregularities.

On the 27th the pulse* was 88 at 6 a.m. ; 57 at 8 a.m. ; 53 at 10 ; 86 at noon ; 51 at 2 p.m. ; 92 at 4 ; 86 at 6 ; 52 at 8 ; 82 at 10, and 54 at midnight. At 2 p.m. the record states "pulse very irregular." Temperature 98°-99°.

December 28th. Slight nausea and vomiting occasionally. Pulse weak and irregular. Sphygmograms taken in the afternoon indicate the same irregularities as occurred on the 29th. The attacks of irregularity did not last long. Strychnine 1/30 grain every four hours.

* We attach no importance to these numbers as indicating the true rate of the heart, as it is quite impossible to determine the pulse rate in the type of irregularity shown by the sphygmograms. The numbers given, however, indicate periods of irregularity at 8 a.m. ; 2 p.m. ; 8 p.m. and midnight ; while the intervening counts indicate a return to the normal.

December 29th, 8'3. Pulse very irregular at 8'30, but regular at 9'15. In the afternoon pulse tracings were taken every hour. At 1'45 great arrhythmia; 2'45 pulse regular; 3'45 irregular again; 4'45 irregular; and this persisted through the rest of the day.

From December 30th onwards nausea and vomiting stopped, and general improvement. Periods of irregularity became less frequent, and confined almost entirely to the night time. The patient says she wakes up with a start, and heart becomes very irregular at once.

On January 11th, 1902, the heart was very irregular during the night, but afterwards remained regular again until, on January 18th, she was discharged from the hospital and reached home in the evening; very irregular during the following night. Patient states that she first noticed the irregularity of the heart 20 years ago during an illness, and since then it has occurred whenever she was ill, excited or exhausted. Vomiting, to which she is occasionally subject, is always followed by heart irregularity. The tendency to irregularity seemed to increase about ten years before the operation, as she observed that smaller occasions gave rise to it than previously. For two years after the operation she suffered very little from her heart, but then some over-exertion gave rise to cardiac irregularity again, and for the last year very slight exertion or exposure to extreme heat or cold induces an attack.

The examination of the heart by the usual clinical methods, during the stay of the patient in hospital, gave no clue to the cause of the irregularity. No enlargement of the cardiac dulness could be made out nor were there any murmurs present, and no objective signs of heart failure were present apart from the irregularity.

Analysis of the Sphygmograms.

The pulse tracings were taken with Jacquet's sphygmochronograph recording $1/5$ secs. The tracings obtained during the irregular periods were all of the same type, and may be represented by some of those taken on December 29th. The marker above the tracing records the time in $1/5$ secs., while the intervals between successive pulses are given below in hundredths of a second.

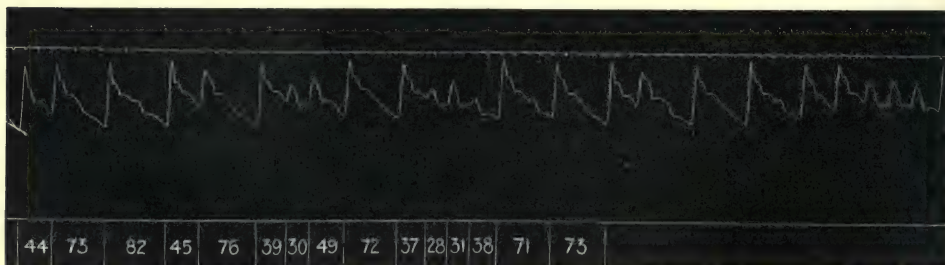


Fig. 1.—Pulse tracing at 1'45 p.m., December 29th.

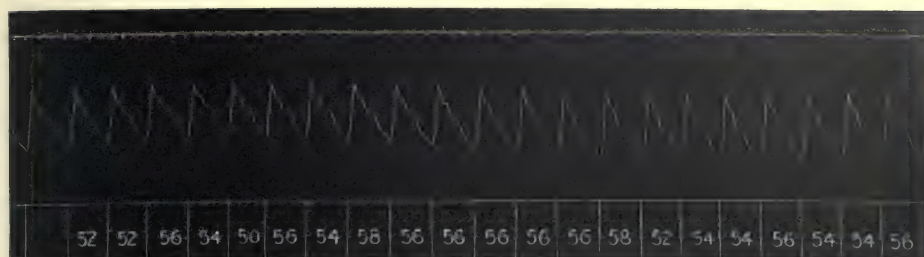


Fig. 2.—Pulse tracing at 2:45 p.m., December 29th.

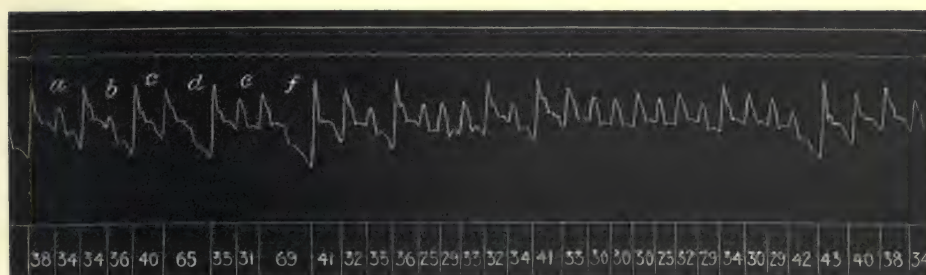


Fig. 3.—Pulse tracing at 3:45 p.m., December 29th.

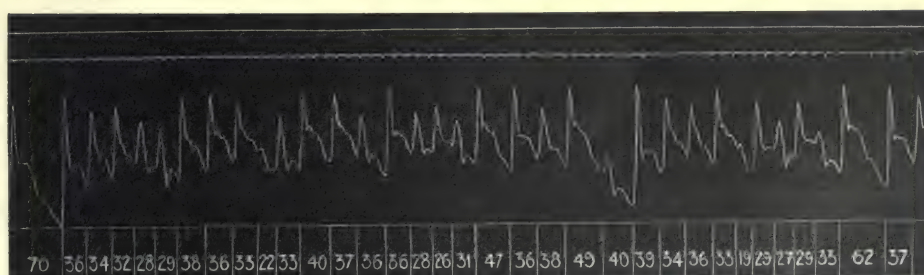


Fig. 4.—Pulse tracing at 4:45 p.m., December 29th.

Of the four tracings, those taken at 1.45, 3.45 and 4.45 p.m., indicate marked and continuous irregularity, while the intervening one at 2.45 presents a quite satisfactory, regular pulse of about 88 per minute. This rate is considerably above the average for the age of the patient, but is not to be regarded as abnormal.* The slight variations in the intervals between the pulsations are not greater than those observed in normal persons. Auscultation at this time indicated no abnormality in the heart sounds, and, in short, the heart and circulation at this time appeared perfectly normal.

The tracings taken before and after this period of regularity offer a marked contrast, the pulse showing great arrhythmia and great variations in the strength of the beats. The general character of the tracings shows a number of large pulsations occurring at irregular intervals, and having interspersed among them smaller pulsations sometimes singly, at other times in twos or threes or in groups of larger numbers. Examination shows that the large excursions of the lever are not so simple as they appear at first sight. For example, in Fig. 1, the descent following the second and third rise of the lever is interrupted by oscillations which are obviously due to a beat of the pulse in each case too weak to make a more definite record. This is perhaps better shown in Fig. 3, in which at *a* and *b* the large excursion of the lever is followed by a weaker contraction, while at *d* the descent of the lever only shows a slight oscillation, yet there can be no question that there was here a secondary contraction, similar in character to those at *a* and *b*, but so weak that it failed to cause even such a beat as is recorded in *b*. In the interval *c*, on the other hand, there was probably no interposed systole of the ventricle. When the stethoscope was applied over the heart there was found a duplication of the first sound in such intervals as have been recorded at *a*, *b* and *d*, while there was no such duplication at *c*. At *e*, Fig. 3, two small elevations follow each other, and at *f*, a third one occurred which was too feeble to do more than arrest the fall of the lever for a moment. A little later, in Fig. 3, a succession of eleven rapid weak contractions are seen. Counting these weak and almost unrecorded contractions, the pulse is found to be beating in the irregular periods at 140-150 a minute. On auscultation the heart sounds were found to be very much more

* Mackenzie. The study of the pulse, p. 53.

rapid than the pulse taken by the finger which, as has been stated, was given as about fifty a minute during the irregular periods.

Even when these small beats are taken into account, the rhythm of the heart is very irregular; for example, during a succession of rapid contractions in Fig. 3, the interval between successive pulses varies from 0.25 sec. to 0.41 sec. There exists no such "regular irregularity" as has been described under the terms *Pulsus bigeminus* or *trigeminus*. In these one normal pulsation is followed regularly by one or two movements of smaller dimensions, but in our tracings no such sequence is to be made out.

Even more striking than the irregularity in the pulse rhythm in this case is the marked variation in the strength of the beats. This variation in strength is of course a very common phenomenon in irregularity of the heart. In the numerous tracings published of late years by Wenckebach, Mackenzie, Cushny and others, this variation in strength is universally noted. But in most of these the strength of the beat varies directly with the length of the preceding interval; the ventricle accumulates more energy during a long interval, and, at the same time, receives more blood from the auricle, and these two factors together cause a very powerful contraction and a very large output of blood. In this case, however, the size of the pulse bears no relation to the length of the preceding interval. It is true that when there has been a great fall in the pressure from the absence of or the inefficiency of the preceding beat, the next contraction is often very large, but this is obviously not the determining factor in most instances, for a strong contraction is often preceded by a shorter interval than a smaller one, and, in other cases, two intervals of equal length are followed by pulsations of very different height. The variations in the size of the pulse must therefore be due not to different degrees of recuperation in the contracting power of the ventricle, but rather to variations in the amount of blood which it receives during its diastole from the auricle. The amount of blood entering the ventricle may be altered by a variety of causes. One condition which occasionally causes pulses of different sizes is where the ventricle and auricle are beating at different rates. For example, if the auricle beats three times to the ventricle's twice, one of the auricular beats discharges no blood into the ventricle, because the auricle contracts against the contracted ventricle. The next pulse is correspondingly

small. This condition, however, leads to a rhythmical variation in the strength of the pulse, as has been shown by one of us in the case of certain poisons which lead to a ventricular rhythm independent of and different from that of the auricle.* No such rhythmical variation in the size of the pulse is to be made out in this case, so that the condition is obviously different from that occurring in animals under these poisons. An irregular and intermittent beat of the auricle might, however, cause the occasional discharge of blood into the ventricle during the diastole, and thus lead to a large pulse wave from the next ventricular contraction; the ventricular rhythm must then be much more rapid than that of the auricle, and, of course, must be developed independently. Another factor which may determine the variations in the strength of the pulse in this case is imperfect diastole of the ventricle. The blood can flow from the auricle only when the intra-auricular pressure is higher than the intra-ventricular, and, if the ventricle remains half-contracted during the diastole, the inflow must be small and the next pulse correspondingly low. If this be the correct explanation of the variations in the size of the pulse, these are, of course, due to the ventricle alone. The auricle might be beating at the same rate as the ventricle, but unable to empty itself against the half-contracted ventricle, or it might be entirely inactive, the blood simply entering the ventricle from the auricle through the pressure exerted on it by the right heart.

Which of these two factors determines the variations in the size of the pulse we are unable to determine from the evidence of the tracings. If the venous pulse had been registered simultaneously with the arterial, much light would have been thrown on the point, and it would probably have been possible to decide whether slow, intermittent, irregular contractions of the auricle are the explanation, or variations in the degree of relaxation of the ventricle. In the absence of such evidence, the latter appears the preferable. It is a feature familiar to everyone who has watched an irregular heart in the living animal, while it is extremely rare to find the auricle beating so much more slowly than the ventricle as would be indicated by the number of full pulses.

The regular beat of the ventricle which is registered by the sphygmograph in normal persons follows an impulse which originates in the "rhythmic area" around the great veins of the heart, and is propagated

* *Journal of Physiology*, xxv., p. 49.

to the ventricle by way of the auricle. Two factors are thus involved directly; the discharge of the impulse from the rhythmic area must be regular, and its path through the auricle unobstructed; and the ventricle must always be in such a condition that it can respond to the stimulus, while its irritability is too low to permit of its initiating a contraction spontaneously. Irregularity of the heart arises either from irregularity in the discharge of impulses or from an abnormal condition of the ventricle which leads to spontaneous contraction before the descending impulse reaches it; in rarer cases the ventricle is unable to respond to the descending impulse and a beat lapses. Careful examination of the pulse often determines whether the irregularity arises from ventricular disability or from the irregular action of the originating area.* For in intermittent pulse due to ventricular lesion the intermission is almost exactly twice the interval between two normal beats, while, if the discharging area be at fault, the intermission is considerably shorter. The fact that the intermission due to ventricular abnormality must be a multiple of the normal length of a pulse wave, follows directly from the consideration that the impulses descending through the auricle are undisturbed in rhythm, and the intermission can end only on one of these reaching the ventricle and originating the first normal beat after the irregularity. That the intermission is shorter when it is due to an irregular discharge from the rhythm-giving area was first deduced from animal experiments, and has been found to conform with clinical experience in too many cases to permit of further doubt.

In our case of irregularity there is no reason to suppose that the ventricle failed to respond to the impulses it received. The length of the apparent intermissions or of the periods of short quick pulses is not a multiple of the single beats. For example, in Fig. 1, the first single beat is 0.44 sec. in duration, and if the next intermission had been due to the failure of the ventricle to contract on the next regular stimulus its length would have been 88 instead of 73. Similar reasoning in each case shows that the irregularity is due, not to variations in the ventricular receptivity of stimuli, but to an irregular discharge of impulses or to an irregular transmission through the auricle or auriculo-ventricular fibres.

* Cushny. *Journal of Exper. Med.*, iv., p. 327.

Mackenzie. *loc. cit.*, p. 297.

Wenckebach. *Ztschr. f. klin. Med.*, 1899, xxxvi., p. 181.

The question arises whether these irregular impulses originate from the same point as those in the normal regular pulse, or whether the irregularity of the discharge is due to the fact that some other point takes up the function, and, being only imperfectly adapted to it, issues only irregular impulses. The latter view has been suggested by Mackenzie,* who holds that in certain forms of continued irregularity the ventricle, or the auriculo-ventricular fibres, assume an automatic or endogenous rhythm, while the auricle may assume the ventricular rhythm, or may remain quiescent or "paralysed." Mackenzie supports this view by a careful analysis of his tracings, and, in the particular cases observed, the interpretation may be correct, although an alternative explanation might possibly suffice without the necessity of adopting the inversion of the cardiac rhythm postulated by him. But in animal experiments, in which the ventricle has been induced to take up an endogenous rhythm by means of drugs, no such irregularities of the heart rhythm are observed,† and, in numerous unpublished experiments in which rapid induced shocks were applied to the ventricle, one of us was able to invert the cardiac rhythm, the ventricle giving the rhythm to the auricle, without rendering the rhythm of either irregular after the first few seconds. An endogenous ventricular rhythm may therefore be perfectly regular in the mammalian heart, and it cannot be assumed *per se* to be the explanation of irregularity in the tracings under discussion.

In our tracings, then, there is nothing to suggest that the ventricle originated the rhythm anywhere; although we cannot exclude the possibility of the whole phenomenon being due to endogenous ventricular influence, it seems rather to have merely responded to the impulses which it received at irregular intervals from the auricle. Not infrequently, the impulses followed each other so rapidly that the ventricle could not dilate sufficiently in the interval to permit of the entrance of blood from the auricle. In other cases blood failed to enter in quantity, although there seems to have been sufficient interval, and here the ventricular contraction caused a very small pulse wave.

The condition of the auricle and rhythmical area in the irregular periods appears to have been one of extreme activity if, as we believe, the rhythm in this case was given to the ventricle by some other part of

* Study of the pulse; *Brit. Med. Journal*, March 5th, 1904.

† Cushny. *Journal of Physiology*, xxv., p. 49.

the heart. This impulse-emitting area was obviously in a state of great irritability, for the impulses amounted to 150 per minute, and at the same time the rhythm was extremely irregular.

The sudden onset of irregularity and its temporary character suggest that while there may be cardiac conditions favouring irregularity, the actual change from the regular beat must be due to affection of other organs. And the fact that it accompanied the nausea and vomiting, and tended to recur when no exertion had been made, and especially in the night time, suggest that the immediate cause of the irregularity was some abnormality of the central nervous system acting on a damaged heart. We are aware that the tendency at present is to regard such conditions as of peripheral origin, and to exempt the central nervous system from responsibility in such phenomena. At the same time, the history of the case inevitably suggests this view, and we hope in the following pages to show indisputably that the central nervous system is capable of giving rise to very marked abnormalities of the heart action.

Animal Experiments.

In the course of the long series of experiments and demonstrations on the dog's heart carried out in the pharmacological laboratory of the University of Michigan, during the last ten years, it has happened occasionally that on opening the chest the heart was found to be beating very rapidly and irregularly. Doubtless other workers in this field may have had similar unfortunate experiences, but we are unaware of any recorded cases. Our dogs were anæsthetised with morphine 0·2-0·3 G. hypodermically, and chloretone administered by the stomach tube. The operation consisted in performing tracheotomy, prolonging the median incision to the lower end of the sternum, sawing through the sternum along its whole length and hooking the two sides of the chest apart, thus exposing the pericardium, which was opened. The myocardiograph was then applied to the ventricle and auricle, and tracings taken on a kymograph with smoked paper. The anæsthesia was invariably deep enough to prevent any manifestation of pain, and the eyelid reflex was absent before the incision was made. Sometimes, however, spontaneous respiratory movements returned while the sternum was being cut through, or, if these were present before, they became quickened and deepened.

And it was soon noted that when this change occurred, the tendency to cardiac irregularity was much greater than in those experiments in which more profound anæsthesia had been induced. Attempts were made to record the changes in the pulse during the operation, but in the experiments in which this was done, the irregularity was not developed. In several cases, however, it was noted that the heart rhythm was normal before and during the first part of the operation, but, that when respiratory movements were induced by cutting through the sternum, the pulse, which had previously been of the usual slow vagus type seen in the dog, suddenly became accelerated and irregular. On examination of the heart in those cases, before the apparatus was applied to it, the ventricles were found in rapid, irregular movement; the relaxation was often very imperfect between three or four successive contractions, and then more complete for one or two beats. The impression was given that the ventricles were responding to a very rapid series of impulses which prevented their diastole, and that they could only relax when their irritability was reduced by fatigue, and then the more complete diastole followed. The auricles were widely dilated, and no systole occurred in them; they were not wholly paralysed and inactive however, for, on close inspection, the fibres proved to be in a state of continual inco-ordinated contraction, each part of the auricle undergoing continuous fibrillary contraction independent of all the other parts. The heart was, in fact, in the condition known to physiologists as auricular delirium or fibrillary contraction in the auricle.

Auricular delirium is not infrequently produced by poisonous doses of bodies of the digitalis series,* and is very readily induced by rapid induction shocks passed through the auricular walls in animals. Several papers† have appeared recently dealing with the subject from the laboratories of Berne and Brussels, and we also have devoted some attention to it. In this condition, the ventricle beats much more rapidly than normally, and the beats are irregular in rhythm, and vary in the degree of systole and diastole. Stimulation of the vagus, in our experience, which coincides with that of Kronecker and Spallitta, has no effect

* Cushny. *Journal of Exp. Med.*, ii., p. 233.

† Kronecker and Spallitta. *Arch. Internat. de Physiologie*, ii., p. 223.

Philips. *Ibid.*, ii., p. 271.

Fredericq. *Ibid.*, ii., p. 281.

whatever on the movement of the auricles, while the ventricle is rendered slower and more regular or may be arrested completely. Philips believes that the fibrillation of the auricle is lessened by vagus stimulation. (It may be mentioned that the tremor in the tracing of the auricle during delirium often appears less marked under vagus stimulation, but this is due to the fact that the ventricle is slower and communicates less movement to the auricle; the fibrillation of the auricle causes no movement of the lever in itself.) The more regular contraction of the ventricle under vagus stimulation is obviously due to the fact that the impulses from the auricle pass less readily through the block; when they are altogether excluded by section of the auriculo-ventricular band, the ventricle beats regularly (Fredericq.) The pulse was recorded by Philips as well as the movements of the ventricle and auricle, and during the auricular fibrillation it is accelerated and very irregular, as we have also observed in our experiments. See Figs. 5, 6.

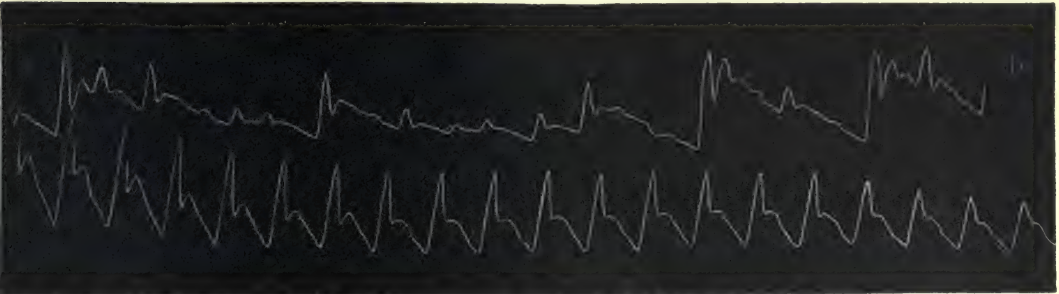


Fig. 5.—Tracings (Hürthle's tonometer) from the carotid of a dog. The lower tracing is the normal; the upper one was taken during auricular fibrillation from rapid electrical stimulation of the auricle.

Stimulation of the dog's auricle with rapid induced shocks causes fibrillary contractions, which pass off when the current is shut off, or very soon afterwards. On repetition several times the interval between the cessation of stimulation, and the return of the normal auricular contractions, becomes longer, until finally it may continue for an hour or more after the electrodes have been removed. Such cardiac depressants as chloroform have no remedial effect, even when pushed until the ventricle almost ceases under them. The only way in which we have been able to

restore the auricle to regular contraction in persistent fibrillation—and this is not successful except occasionally—is by the application of cold directly to the heart. Large quantities of chilled salt solution poured on the exposed heart sometimes restores it to regular contraction.

The auricular delirium, which we have described as occurring in dogs during the operation, was obviously the result of sensory stimuli carried from the chest wall to the central nervous system. This might affect the heart either by inducing changes in the blood pressure (shock) or by nervous

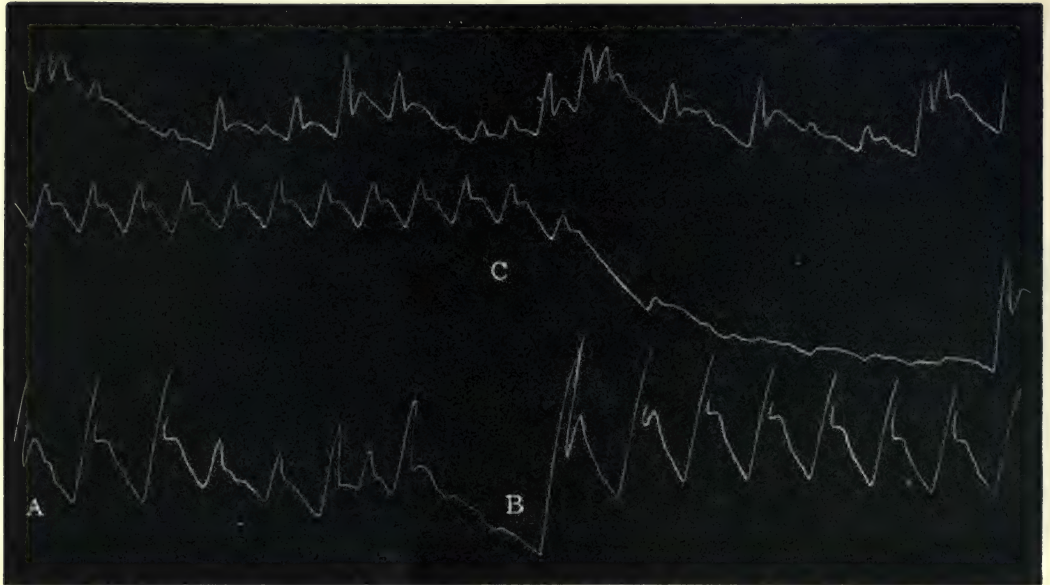


Fig. 6.—Tracings (Hürthle's tonometer) from the carotid of a dog. From *A* to *B* in the lowest tracing the auricle was stimulated with a very weak tetanising current, and imperfect fibrillation resulted at intervals. From *B* onwards, the stimulation ceased. In the middle tracing a stronger series of shocks was applied to the auricle at *C*, and it immediately passed into fibrillation. The highest tracing was taken during prolonged fibrillation from auricular stimulation.

impulses passing along the cardiac nerves to the heart. In the descriptions of shock given in surgical textbooks, the heart is often said to be irregular, but personal enquiry among our surgical friends failed to elicit any information in regard to the character of the irregularities, and we were led to the conclusion that cardiac irregularity in shock is extremely

rare or only seen in moribund cases. We are therefore disposed to attribute the condition observed in our experiments to reflex influences on the heart. These impulses must reach the heart either by the vagus or by the sympathetic fibres. And, as a matter of fact, we have observed one instance in which vagus stimulation was followed by delirium. In this dog, the heart was peculiarly irritable and much accelerated. The myocardiograph had been attached to the right ventricle and auricle, and a tracing was being taken. The effects of electrical stimulation of one vagus in the neck were being demonstrated, and the usual response followed—arrest of the auricle and ventricle in diastole. But when the current was shut off, the heart, instead of returning to its normal rate, passed into fibrillary contractions in both auricle and ventricle. This is the only instance in which we have seen the unpoisoned heart affected in this way from the cessation of inhibition. In several experiments in which the irritability of the heart was exaggerated by large doses of members of the digitalis series or of barium, section of the vagus led immediately to fibrillary contractions in the auricle and ventricle.

As has been stated, we have obtained no tracings of the pulse in dogs in which the heart passed into auricular delirium during the operations. The phenomenon is a rare one in our experience, although we have observed it sufficiently often to have no doubt as to its occurrence, and as to its association with the operation. In several experiments the pulse was recorded during the operation by means of Von Frey's or Hürthle's tonometer, attached to the carotid artery, and the anæsthesia was maintained at a point at which, while pain was absent, the reflexes were retained as far as possible. In none of these, however, was the auricular delirium developed, although the pulse was much accelerated; occasionally intermissions of the pulse occurred of the auricular type, indicating perhaps a tendency to fibrillation. The acceleration was very slight, or entirely absent in experiments in which atropine had been previously injected in quantities sufficient to paralyse the cardiac vagus. The acceleration in dogs without atropine was thus due for the most part to lessened vagus tone, from the centre being inhibited by impulses from the seat of operation. It seems likely that the auricular delirium observed in rare cases in dogs during the operation is similarly to be explained by the disappearance of inhibition; the auricles must in these cases, however, be in some quite unusual condition of irritability.

Of course we cannot claim to have shown definitely any connection between this type of irregularity in the dog's heart and that in the case described. At the same time there exist similarities between them, and the sudden onset of the irregularity in each suggests a common cause; in the dog the lesion acts through the central nervous system, and the history of the patient suggests that here also the irregularity was of central origin. We had hoped to have the opportunity of continuing the investigation in other cases of irregular heart, but as circumstances preclude our working further together, we have decided to put this imperfect investigation on record, in the hope that others may be interested in the suggestion made, and may be able to prove or disprove its correctness.

Summary.

A case of paroxysmal arrhythmia, with marked acceleration of the heart, is described, and it is shown that the irregularity is due to irregular discharge of impulses and not to defects in the contraction of the ventricle, which appears to respond to the impulses received. A similar form of irregularity in the dog's heart is described, and it is shown that this is due to the ventricles receiving irregular stimuli from the auricle, which is in a state of fibrillation. This form of arrhythmia is shown to occur in the dog occasionally from peripheral irritation, and it is probable that this gives rise to reflex inhibition of the vagus centre, which, acting on an abnormal heart, causes auricular fibrillation. The suggestion is made that in the patient described, and in other cases of paroxysmal arrhythmia, the condition is due to auricular fibrillation, possibly from inhibition of the vagus centre.

RESEARCHES ON CERTAIN PROBLEMS OF
PLAGUE IMMUNITY.

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Researches on Certain Problems of Plague Immunity.

THE object of the present communication is to record the results of certain researches on Plague Immunity which have been carried out in the Plague Department of the Lister Institute.

The chief problems dealt with in this contribution may be arranged as follows :—

1. Does the *B. pestis* produce a toxin capable of demonstration and suitable for experimental purposes ?
2. If such a toxin is obtainable, does its injection into the animal body result in the production of an antitoxin, and in how far does the ordinary anti-plague serum, which undoubtedly confers protection on such animals as rats and mice, owe its immunising property to an antitoxic action ?
3. By what mechanism does an anti-plague serum exercise its anti-infectious effect ?

It is unnecessary here to enter into all the details of the methods of preparing anti-plague serum, but for the sake of clearness in discussing certain of the questions connected with the above problems it seems desirable to give a brief outline of the subject.

As will be readily understood the most rigid precautions are necessary to obviate the risks of infection during the process of immunisation.

The ordinary procedure for obtaining an anti-microbial serum is employed. The animals first undergo a course of intramuscular, or intravenous, injections of killed cultures of the bacilli, till a certain degree of immunity is acquired. In the case of the plague bacillus this preliminary stage in the immunisation occupies a considerable period, extending from four to six months, or even longer, the slow absorption of the œdematous and indurated swellings which result from the subcutaneous and intramuscular inoculations being chiefly responsible for

the prolongation of the process. On the other hand, considerable risks of thrombosis, causing sudden death, are attached to the intravenous injection of killed cultures. This danger appears to be much greater with killed than with living cultures, and is probably due to the setting free by the heating to which the culture is subjected of a ferment or of nucleo-protein.

The further stage in the process of immunisation consists in giving the animal a series of intravenous injections of living virulent bacilli. The general experience has been that the injection of even a small quantity of living culture greatly increases the protective action of the serum.

Roux and Dujardin-Beaumetz at the Pasteur Institute Paris, and Calmette at the Lille Institute, prefer intravenous injections throughout, and use bacterial emulsions, prepared from agar cultures, for both the early and later stages, whereas Tavel Krumbein and Glücksmann at Berne use old broth cultures, killed by heating to 65-70° C. (Haffkine's Prophylactic Fluid), injected subcutaneously or intramuscularly in the early stages, and young broth cultures injected intravenously in the later stages of immunisation.

When "anti-plague serum" is referred to in this paper, a serum prepared by one or other of these methods is indicated. In certain of the following experiments the serum used was from animals which had been treated with heated or unheated filtrates only.

Brief Review of the Literature on Plague Toxin.

Yersin, Calmette and Borrel (1895) stated that the filtrates from broth cultures of the *B. pestis* have no toxic action on experimental animals, but that emulsions from agar of the bodies of the bacilli, which have been killed by heating to 58° C., cause the death of guinea-pigs and rabbits when injected subcutaneously or intraperitoneally.

Roux is stated by Metchnikoff (1897) to have obtained very powerful plague toxins by employing an organism, the virulence of which had been elevated by animal passage in collodion sacs. The filtrate from a bouillon culture, precipitated by ammonium sulphate, gave a toxin of which the lethal dose for a mouse was one-fourth of a milligramme, for a rabbit four centigrammes. The guinea-pig is the most

resistant of the usual laboratory animals. The toxin is destroyed by a temperature of 70°C .

Lustig and Galeotti (1897) obtained toxic substances by extraction with a one per cent. solution of caustic potash, and subsequent slight over-neutralisation with a one-half per cent. acetic acid solution. The resulting precipitate is highly toxic, and is employed as a vaccine.

The German Plague Commission (1899) points out that certain animal experiments indicate that the toxic action is not an important feature of the pathogenic effects produced by the plague bacillus. They refer to the condition observed in monkeys, in which a great part of the body is affected with oedema, and where the exudate swarms with bacilli, and yet in these animals strikingly few symptoms of toxæmia are displayed.

Notwithstanding this view of the Commission, it cannot be denied that toxæmia is a marked feature in many cases of human plague. In a considerable proportion of all cases the lesions remain localised in the affected lymphatic glands, no bacilli being present in the blood-stream, and yet these cases die with the symptoms of profound toxæmia. It is certain that the pathogenic effects of the plague bacillus are the result of toxic action.

The English Plague Commission (1898-99), in speaking of the subject of toxin, states, "We, for our part, have tested the effect which is exerted by heat upon the filtrate from a plague culture. It results from these experiments that the soluble toxins which are, under certain conditions, developed in the fluid nutrient medium in which the plague bacilli are grown, are apparently not affected by exposure to a temperature of even 100°C ."

The Austrian Plague Commission (Albrecht and Ghon, 1900), obtained results in the main similar to Markl's, to be described directly. Markl's work was done in part with the Commission. They obtained toxic filtrates from broth cultures, the toxicity of which increased with the age of the culture up to a certain period, two to three months, after which no increase took place. The filtrates from cultures even only five days old were toxic, and the toxins differed only quantitatively from those obtained from older cultures.

The bacillary bodies, from cultures on agar, which had been killed by heating to a temperature of 55° - 60°C ., produced toxic effects similar to those observed with the filtrates. (115)

The toxic filtrates were, in their experience, fairly stable, but 20 minutes in water at a temperature of 100° C. completely destroyed the toxicity for mice.

By means of injections of the filtrates they were able to obtain evidence of active immunity against toxin, "Giftfestigkeit," and the animals which had acquired this showed also some increase of resistance to bacterial infection.

Markl (1898 and 1901) concluded, from a large number of experiments, that the plague bacillus, when grown in bouillon, under aerobic conditions, is capable of producing soluble toxins. The bacillus is grown in bouillon, in flat flasks, to allow a free supply of oxygen, since, under anaerobic conditions, no toxin formation takes place. Though toxin may be demonstrated in a very short period, even within 24 hours, two months at room temperature are required for the maximum toxicity of the cultures to be attained. The optimum temperature for toxin production is room temperature; 37° C., while admitting of toxin formation, is less favourable, a circumstance due partly to the injurious influence on the bacillus of the higher temperature, partly to its deleterious action on the already formed toxin. This susceptibility of the toxin to the action of heat is a marked feature; a temperature of 70° C., for 15 minutes completely destroys the toxicity of the filtrate for mice, whereas it still remains toxic for rats, rabbits and guinea-pigs. From this observation Markl argues that the filtrates contain two different, but related, toxins. The relationship is best shown by the fact that animals which have been injected with heated filtrates furnish an antitoxic serum which is capable of protecting mice against the unheated toxin.

Markl believes that, by injecting goats and horses, with toxin, an antitoxin may be obtained, and he suggests the advisability of employing an anti-plague serum, obtained by a combined method of immunisation with bacilli and with toxins.

He regards the toxin as a product of genuine bacillary secretion, and not merely as the result of the degeneration and death of the bacilli, with subsequent soaking-out of intracellular poisons. He bases his opinion on a number of observations:—First, the toxin is demonstrable at a very early period in the history of the culture, when the number of dead and degenerated bacilli is too small to account for the toxin present; second, the toxin production bears a certain relationship to the

virulence and habit of the culture. To obtain powerful toxins, the bacillus must have been recently isolated from the animal body. After a period of cultivation on artificial media, and especially if high temperatures have been employed, it loses its toxigenic property, which may, however, be recovered in part at least by fresh passage through the animal body.

Kolle (1903), in proceeding to study the production and nature of plague toxin, selected for his experiments such cultures as, from their behaviour in the animal body, might be expected to prove toxigenic on artificial media. He observed that, after a large number of passages through the rat, plague cultures showed a tendency to lose their property of multiplication in the blood, tissues, and organs of the animal body. The death of the animals resulted almost entirely from the toxins produced at the site of inoculation and in the neighbouring buboes. In other words, the cultures, before their frequent passage, tended to produce a plague septicaemia, after their passage a plague toxæmia. The toxæmic symptoms came on at an early period, when the multiplication of the bacilli in the rats was occurring solely at the site of inoculation. Kolle employed an alkaline bouillon.

Whatever the nature of the flask employed for the cultivation of the bacillus provision was made for an abundant supply of oxygen by having only a moderately thin layer of medium at the bottom of the vessel. Occasionally a little blood from the rat or rabbit was added to the bouillon, in which case the culture was observed to be very abundant. The cultures were grown some at 37° C., others at 35° C., others at 22° C., and still others in the ice-safety at from 6° - 10° C.

A number of methods were employed to obtain the toxin, apart from the bacilli. By the addition to the cultures of phenol and toluol, and subsequent centrifugalisation, a sterile supernatant fluid was obtained which was tested for toxicity. The Chamberland filter was also employed, the filtrate being tested for sterility, as in certain cases the plague bacilli have been found to pass the filter.

Kolle employed for his experiments rats of 180-200 grammes weight. In his experience the guinea-pig and rabbit are too resistant, while the mouse gives irregular results or proves too susceptible. Intraperitoneal injection was in every case employed.

In young cultures, of from 3 - 4 days, no toxin could be demon-

strated in the filtrates. On the other hand, if the bacillary bodies were present, the cultures had a markedly toxic action. From this it must be deduced that the toxin is bound to, or more probably contained within, the bacillary bodies. Such toxins have an incubation period of 6 - 8 hours, death usually occurring within 24 hours. The symptoms are those of collapse and giddiness, and point to the action of a brain and heart poison. The common effects of a bacterial disease are manifested in the spleen, bone-marrow and liver, viz., fatty degeneration and coagulative necrosis. The peritoneum is injected and may present small hæmorrhages, which may also occur in other organs. Kolle regards this as the primary specific plague toxin, since in the human subject similar effects are not infrequent. In animals which survive the acute toxic effects marasmus may occur.

In older cultures of from 7 - 10 days, where the growth has taken place at 37° C., an increase in the toxicity of the filtrates, and in the character of the resulting intoxication, can be determined, whereas the filtrates from cultures at lower temperature produce effects similar to those obtained from cultures 1 - 2 days old at 37° C. The older the culture, up to a certain point, the higher is the toxicity. The cultures at 30° - 37° C. reach their maximum toxicity in 3 - 4 weeks, whereas those at lower temperatures require 8 - 10 weeks.

The toxic action of the filtrates from the older cultures is characterised by a shortening of the incubation period. Symptoms of intoxication occur in 10 - 15 minutes, and in the case of the larger doses death may occur in 1 - 2 hours. The most prominent of the symptoms produced by these filtrates from old cultures are convulsions, from which the animals may recover. These effects are produced by non-specific substances of the nature of cadaverin and putrescin, which have also been demonstrated in old cultures of *B. pyocyaneus*, *B. typhosus* and *B. cholerae*. Associated with these substances, however, there are present in the old cultures specific toxins which may or may not be identical with those occurring in young cultures.

Kolle holds that the specific toxic substances originate not from a secretory process on the part of the bacillus, but are produced by the death of the bacilli with subsequent soaking-out of the intrabacillary toxin.

Such intra-cellular toxins never give rise to the formation of anti-

toxins. Kolle tested various anti-plague sera for antitoxic properties with negative results. He found that normal horse serum possessed the property in some degree, and that the slight toxin-neutralising properties of sera obtained by immunisation did not exceed the limits found in normal serum.

He attempted to immunise horses by intravenous injection of the primary toxin from young cultures, in others he employed the toxins from old cultures, but in neither case could any antitoxic property be demonstrated in the serum.

The serum and toxin were mixed, and injected peritoneally. Where 4 lethal doses of toxin were employed, 8 rats so treated all died. When 2 and 3 lethal doses of toxin were employed, a certain number of rats recovered, even when normal serum was used. Thus, out of 16 rats injected with a mixture of plague serum and toxin, 9 died; out of 8 injected with plague toxin and normal serum, only 5 died.

Kolle therefore concluded that the toxins found in plague cultures were intracellular toxins, and that consequently, in accordance with a general biological law, their injection into the animal body does not stimulate to antitoxin production. The question of the tenability of this so-called "general biological law" will be discussed later.

The three following investigations are grouped together because, in all, the toxic or immunising substance, is derived from the animal body.

Terni and Bandi (1900), proposed to employ, as an anti-plague vaccine, the peritoneal exudate obtained from guinea-pigs and rabbits which had been infected intraperitoneally, and killed during the death agony. The fluid, after being tested to ascertain that it was free from organisms other than the *B. pestis*, was incubated for 12 hours, and then submitted to a temperature of 50° - 52° C. for two hours. The fluid was brought to a suitable volume by means of a weakly alkaline normal salt solution, containing 0.5 % phenol. The authors claimed that good immunity was obtained, even in 4 to 5 days, and that the injection did not aggravate an already existing infection, but rather tended to prolong life. The blood of an individual who had received an injection of 1.5 to 2.5 c.c. showed, within 8 to 10 hours, a hindering influence on the development of the *B. pestis*.

Hueppe, F., and Kikuchi, Y. (1905), starting from the work of Bail, Kikuchi and Weil, propose to employ, as an immunising agent, the

peritoneal exudates obtained from guinea-pigs infected in the peritoneum with *B. pestis*. By means of two prophylactic injections of this fluid or "aggressin," they succeeded in conferring a high degree of immunity on mice, guinea-pigs, and rabbits. The injection of the substance was followed by a period of hypersensibility. The theory of the method and its relation to those of other workers will be discussed at a later period.

Klein (1905), while investigating the vitality of the *B. pestis* in certain organs, spleen and lung, and in the buboes of animals dead of plague, found that though all the bacilli had been destroyed by the process of drying over sulphuric acid, an emulsion of the organs, when injected into mice, was capable of causing the death of these animals within 20 hours.

All the phenomena of acute plague are exhibited, without, however, the *B. pestis* being present in the tissues. When the amount of emulsion injected was insufficient to cause death, the animals, after recovery from the local swelling and constitutional disturbance which resulted from the injection, were found, on being tested at a later period, to be refractory to infection, even when a virulent plague bacillus was injected.

Guinea-pigs, inoculated cutaneously with a bacillus of moderate virulence, develop a sub-acute form of plague, death occurring from the fourth to the ninth day. The lesions found are necrotic buboes, necrotic nodules in the spleen and liver, and particularly necrotic nodules and patches in the lungs. In sections of such organs the central parts of the necrotic nodules and patches are found to be crowded with bacilli, whereas the peripheral parts, consisting of debris, contain few, if any, bacilli. From this it may be inferred that the necrotic changes are due, not to bacilli themselves, but to their toxin.

Klein proposes to prepare a prophylactic substance from such material. The bubo, enlarged spleen, necrotic lung and liver of guinea-pigs which have died from sub-acute plague, are cut out, minced aseptically, and then spread out in thin layers on sterile glass dishes, and dried over sulphuric acid at 46° - 47° C. There is thus obtained "a material which not only can be very easily and rapidly prepared, but which is of a uniform and reliable efficacy, and in every way indeed superior to any of the other prophylactics." Klein calculates that a single large guinea-pig would yield 800-1000 human doses of the new prophylactic.

It is unnecessary here to go more minutely into the results obtained on animals, or the advantages claimed for the use of this material. From our present standpoint, however, the toxicity of the substance is of interest.

(1) It kills a large percentage of mice within 20-24 hours in doses of 1-5 milligrammes.

(2) Prepared from acute cases, it kills 15-25% of half-grown white rats in doses of 5-8 milligrammes; prepared from sub-acute cases, 10-12 milligrammes are required to produce a fatal effect.

(3) 20 milligrammes fail to kill a guinea-pig of 200-300 grammes weight.

Klein holds that the effects produced by the substance are due not merely to the bacilli contained in the material, but depend largely on the presence in the necrotic area of a "tissue toxin."

Besredka (1905 and 1906) has proposed a new method for the preparation of typhoid and plague endotoxins.

The growth from an agar culture of *B. pestis* is emulsified, heated to 60° C. for one hour, and then dried in vacuo. The lethal dose for the mouse of this dried substance, injected subcutaneously, is five decimilligrammes. To obtain the plague endotoxin in the soluble state, the dried bacilli are put in normal salt solution and horse serum in fixed quantities. The mixture is placed in the ice-safe overnight, and is centrifugalised on the following day. The clear supernatant fluid contains the endotoxin, and the deposit of a sticky consistence is formed of atoxic plague bacilli.

The soluble endotoxin is highly toxic for the mouse, *e.g.*, 0.02 centigramme of dried bacilli, to which 1 c.c. of physiological salt solution and 4 c.c. of horse serum have been added, corresponds to about 20 lethal doses of endotoxin.

The plague endotoxin keeps for months in the ice-safe. It is thermostable; heating to 55° C. for one hour leaves it intact; it is slightly attenuated by heating for one hour to 65° C., but it is not destroyed by heating to 57° C. for 15, or even 24 hours.

Its essential characteristic, according to Besredka, is that its action is neutralised by anti-plague serum.

Besredka also investigated the endotoxin of the typhoid bacillus, and

found that 32 lethal doses of the endotoxin are neutralised by 0·2 grammes of the serum of an immunised horse.

Similarly, he found that the serum of horses which had been injected intravenously, either with plague bacilli or with the soluble endotoxin, was capable of neutralising the plague endotoxin.

The Writer's Experiments on Plague Toxin.

The writer's experiments on the subject of Plague Toxin were directed chiefly to the study of the influence of certain factors on the toxicity of cultures of different races of the plague bacillus.

Technique used for obtaining the Toxin.—In certain cases the cultures were well shaken with toluol, the bacilli allowed to sediment, and the bacterial free supernatant fluid examined; but for the most part the filtrates of cultures were investigated. The filtration was carried out by means of a "tandem" filter, *i.e.*, the fluid was first passed through a Berkefeld, and afterwards through a Pasteur-Chamberland filter. The more porous Berkefeld filter removes the coarser particles, and this prevents the blocking of the Pasteur-Chamberland candle which, employed in this way, always gave a sterile filtrate. The loss of toxin is perhaps less by this method than if the Pasteur-Chamberland candle alone were employed, because in the latter case the blocking of the pores by the larger particles tends to make a very close filter. In every case it is desirable to test the sterility of the filtrates and centrifugates.

Temperature.—The temperature most frequently employed for the growth of the cultures was what is generally accepted as the optimum temperature for the growth of the *B. pestis*, *viz.*, about 30° C.

The Medium.—The medium employed was the ordinary bouillon such as is used in the preparation of diphtheria toxin. In most cases it was made neutral or very slightly alkaline to litmus, but, with the object of ascertaining whether toxin formation would be aided by the use of an alkaline broth, in certain cases the broth was prepared as for diphtheria toxin, 7 c.c. per litre of normal caustic soda being added after neutralisation with litmus as the indicator. The results obtained with the filtrates from two flasks of the same broth, in the one case neutral, in the other case alkaline, are shown in Tables I. and II. In this case, the neutral broth appeared to be the more favourable medium for toxin production.

TABLE I.—TOXIN 15.

Filtrate from a 2 month's growth on Neutral Broth.
 Test on Mice of 20-25 grammes weight.
 Subcutaneous injection.

Mouse.	Volume of Toxin.	Result.
1.	·05 c.c.	Lived.
2.	·1 "	Died 48 hours.
3.	·3 "	Died 24 hours.
4.	·5 "	Died 24 hours.
5.	1·0 "	Died 24 hours.

TABLE II.—TOXIN 16.

Filtrate from 2 month's growth of the same bacillus and on the same broth as Toxin 15. In this case the broth was made alkaline, 7 c.c. of normal caustic soda per litre being added after the neutral point to litmus was reached.

Test on Mice of 20-25 grammes weight.
 Subcutaneous injection.

Mouse.	Volume of Toxin.	Result.
1.	·05 c.c.	Lived.
2.	·1 "	Lived.
3.	·3 "	Lived.
4.	·5 "	Died 48 hours.
5.	1·0 "	Died 24 hours.

It was observed that the addition to the broth of 1:4 to 1:8 of normal horse serum heated to 65° C., gave a very abundant growth, in some cases with pellicle formation, but so far as this was investigated no increase in toxin formation could be demonstrated.

The cultures were grown for the most part in large flat bottles each containing 1 to 1½ litres, but Erlenmeyer's flasks, with a thin layer of medium in the flask, were also employed. Previous experience had

shown that under these conditions the supply of oxygen is sufficient to satisfy an organism so exigent in this direction as *B. diphtheriæ*.

Appearance and Age of the Culture.—The cultures vary greatly in the appearances they present. In certain cases the growth begins as minute flocculi in the bouillon, the surrounding fluid remaining clear. Other cultures show an almost uniform turbidity. To certain of the flasks cocoa butter or butter was added, and in these good stalactite formation was observed. No connection between the type of growth and the toxicity could be determined. The culture, as it increases in age, becomes very alkaline, and in two months there is usually a large deposit containing bacterial debris and masses of crystals of triple phosphates.

One change is of considerable significance in relation to toxin formation. A platinum loopful from a culture a few days old, planted on an agar plate, gives an abundant growth, whereas, from a flask two months old, little or no growth may be obtained unless a larger quantity of material be employed for inoculation. In the two months' old culture, almost all the bacilli present have lost the usual morphological characters of the plague bacillus, the deposit in the flask consisting almost entirely of bizarre involution forms or mere detritus.

The Period of Maximum Toxicity.—Some preliminary tests were made on the toxicity of the filtrates from cultures 10 days and two months old in bouillon of the same brew. Such a test shown in Tables III. and IV. indicates that at 10 days, if toxin is present, its concentration is much less than at two months.

TABLE III.—TOXIN 7.

Filtrate from 10 day's growth on Neutral Broth.

Test on Mice 15-20 grammes weight.

Subcutaneous injection.

Mouse.	Volume of Toxin.	Result.
1.	·05 c.c.	Lived.
2.	·1 "	Lived.
3.	·5 "	Lived.
4.	1·0 "	Lived.

TABLE IV.—TOXIN 13.

Filtrate from 2 month's growth on same Neutral Broth as No. 7.

Test on Mice 15-20 grammes weight.

Subcutaneous injection.

Mouse.	Volume of Toxin.	Result.
1.	·05 c.c.	Lived.
2.	·1 "	Died 48 hours.
3.	·3 "	Died 48 hours.
4.	·5 "	Died 24 hours.
5.	1·0 "	Died 24 hours.

It thus appears that the maximum toxicity of the culture is synchronous with the period when most of the organisms have undergone degenerative changes or have died ; it seems probable, therefore, that the toxic substances have been set free as the result of a process of autolysis. Reference will be made to this in discussing the question of toxins. Most of the filtrates tested were from cultures 8 to 10 weeks old.

Great variations were observed in the toxicity of the filtrates from different brews of bouillon made by the same methods and with the same proportions of the components. Similar inexplicable variations are well known in the preparation of diphtheria and tetanus toxin.

It would be impossible here to give the protocols of all the tests carried out in ascertaining the toxicity of different filtrates, but those recorded are typical of the results obtained in the case of the more toxic filtrates. A number examined were found to be much less toxic than Toxin No. 10. As this toxin was employed for a test toxin in testing the sera, the protocols of tests on mice and rats are given (Tables V., VI., VII. and VIII). It will be noted that there is a certain amount of irregularity in the results on mice, which makes the determination of the minimal lethal dose a matter of some difficulty.

TABLE V.—TOXIN 10.

Filtrate from culture on Neutral Bouillon 10 weeks old.
 Tested on Mice of 20-25 grammes weight.
 Subcutaneous injection.

First Test.

Mouse.	Volume of Toxin.	Result.
1.	'01 C.C.	Lived.
2.	'02 "	Ill—recovered.
3.	'03 "	Ill—recovered.
4.	'04 "	Died 8 days.
5.	'05 "	Ill—recovered.
6.	'1 "	Died 24 hours.
7.	'2 "	Died 24 hours.
8.	'5 "	Died 24 hours.
9.	'5 "	Died 24 hours.

TABLE VI.—TOXIN 10.

14 days after previous Test on Mice of 15 grammes weight.
 Subcutaneous injection.

Second Test.

Mouse.	Volume of Toxin.	Result.
1.	'05 C.C.	Died 24 hours.
2.	'1 "	Died 24 hours.
3.	'2 "	Died 24 hours.
4.	'3 "	Died 48 hours.
5.	'4 "	Died 24 hours.
6.	'5 "	Died 24 hours.
7.	'6 "	Died 24 hours.

TABLE VII.—TOXIN 10.

14 days after previous Test on Mice of 15 grammes weight.
Intraperitoneal injection.

Second Test (continued).

Mouse.	Volume of Toxin.	Result.
1.	'02 C.C.	Died 24 hours.
2.	'05 "	Died 24 hours.
3.	'1 "	Died 24 hours.

TABLE VIII.—TOXIN 10.

Testing Toxicity on Rats.
Subcutaneous injection.

Rat.	Weight in grammes.	Volume of Toxin.	Result.
1.	125	'1 C.C.	Died 3 days.
2.	125	'5 "	Died 24 hours.
3.	150	1'0 "	Died 24 hours.
4.	155	2'0 "	Died 48 hours.

It is interesting to note that rats and mice offer much less resistance to the action of plague toxin than do rabbits and guinea-pigs. That the latter animals have the more powerful defence is shown by the experimental results given in Tables IX. and X.

TABLE IX.—TOXIN 10.

Testing Toxicity on Guinea-pigs.

Subcutaneous injection.

Guinea-pig.	Weight in grammes.	Volume of Toxin.	Result.
1.	240	'1 c.c.	Unaffected—lived.
2.	240	'5 "	" "
3.	260	1'0 "	" "
4.	250	2'0 "	" "
5.	250	3'0 "	" "
6.	260	4'0 "	" "

TABLE X.—TOXIN 10.

Testing Toxicity on Rabbits.

Subcutaneous injection.

Rabbit.	Weight in grammes.	Volume of Toxin.	Result.
1.	550	'05 c.c.	Unaffected—lived.
2.	500	'1 "	" "
3.	520	'5 "	" "
4.	526	1'0 "	" "
5.	950	2'0 "	" "
6.	760	3'0 "	" "
7.	800	4'0 "	" "

All guinea-pigs and rabbits alive at the end of one month.

In regard to the stability of the toxin, the writer's experience was in agreement with Markl's. The toxins were found to be rather unstable, especially at ordinary hot weather temperatures as, for example, when Toxin 10 was tested two months after the test recorded in Tables V., VI., VII. and VIII., it was found to have so far deteriorated that the minimal

lethal dose had risen from the 0.1 c.c. of the Table to 0.8 c.c. Higher temperatures, as might be expected, cause similar deterioration in a much shorter time. The effect of exposing the toxin to a temperature of 70° C. is shown below:—

TABLE XI.

Showing influence of heat to 70° C. on Toxin No. 10.

Mouse.	Weight in grammes.	Volume of Toxin.	Result.
1.	20	1 c.c.	Dead in 24 hours.
2.	15	1 "	Dead in 6 hours.
3.	20	1 "	Dead in 8 hours.
		Toxin heated to 70° C. for 30 minutes.	
4.	15	1 c.c.	Lived.
5.	15	1 c.c.	Lived.
6.	20	1 c.c.	Lived.

Where irregularities occur, such as are frequent in the testing of plague toxin, it is extremely difficult to trace the influence of the virulence of the race of bacillus on the toxicity. It was found impossible to establish any definite relation between virulence and toxicity, but a strong impression was formed that as a culture diminished in virulence the filtrate obtained from it became less toxic.

Incubation Period.—The incubation period lasts from 6 to 24 hours according to amount of toxin injected. In certain cases, where very old cultures are used, convulsions occur almost immediately after the injection, but these are probably due to the presence of cadaverin, as has been suggested by Kolle. These frequently pass off in a short time to be succeeded by the symptoms of toxic action.

Effects and Symptoms resulting from the Injection of Toxin.—If the injection is subcutaneous, within 24 hours a soft œdematous swelling, often very extensive, is observed in the vicinity of the site of inoculation.

In acute cases, after the period of incubation, the animal hunches itself up in a corner of the cage, refuses to eat, and, when disturbed, moves in an inco-ordinate manner, as if suffering from alcoholic intoxication. Convulsions are frequent just before death, which appears to be due to collapse.

In chronic cases the early symptoms are less acute, the animal gradually loses flesh till a profound state of marasmus may result. In some of the rats suffering from chronic toxæmia following the injection of toxic filtrates, we have observed a form of paralysis resembling diphtheria paralysis in the guinea-pig. The hind limbs are first affected, then the fore limbs, and ultimately the respiratory muscles may be involved. These animals are often greatly emaciated. Convulsions frequently occur immediately before death.

Post-mortem Changes observed in Animals dying of Toxæmia.—In the animals which die of acute toxæmia, say within 24 hours after subcutaneous injection of a toxin, the most marked feature is œdema round the site of inoculation. This may be very extensive, affecting the whole abdominal wall, and small hæmorrhages are sometimes present. The neighbouring lymphatic glands may be enlarged. Hæmorrhages are sometimes present in the viscera, especially where the injection has been intraperitoneal, in which case the peritoneum and the mucous membrane of the alimentary canal are injected or hæmorrhagic.

In the animals which survive several days or weeks, wasting of the musculature is a marked feature. Fatty degeneration of the liver is a frequent lesion, and necrotic areas are present in a few of the cases. Where death occurs at a late period the spleen may be enlarged.

The Question of a Plague Antitoxin.

With the view of ascertaining whether the injection of toxic filtrates would stimulate the animal body to antitoxin production, two horses were specially employed for immunisation with the filtrates of cultures. In the case of one, the horse "R" filtrates from bouillon cultures, which had been heated to 70° C. for one hour, were alone used. This animal, in the three months preceding the removal of the blood, the serum from which was tested, received injections amounting to 1080 c.c. of filtrates.

The second animal, after a course of injection of heated filtrates, was further treated with injections of filtrates of unheated cultures. In the three months preceding the bleeding from which the serum tested was obtained, nearly two litres of unheated toxin were injected in doses of from 60 to 300 c.c. Among other toxins, nearly a litre of Toxin No. 10, the tests of which have been recorded, was employed in the immunisation.

As already mentioned, the other horses whose sera were tested were immunised by the ordinary methods. The case of the horse "Pr." requires some comment. This animal had been immunised by the Berne method, and had received large injections up to 300 c.c. at one dose of concentrated Haffkine's Prophylactic Fluid. This was followed, as is usual, by a number of intravenous injections of living broth culture. On reviewing the treatment which this animal had received before the removal of the sample of serum tested, it was noted that within the six weeks of treatment preceding the interval which always intervenes between the last injection and the venesection, the animal received the following injections:—One of 40 c.c. and another of 90 c.c. of broth cultures four days old, one of 100 c.c. two days old, and one of 40 c.c. six days old. In regard to this, it is to be noted that these injections were given rather more frequently than usual, and that 180 c.c. of the material was 4 to 6 days old when at least traces of toxin might be expected to be present.

It is well known that out of a number of horses immunised against diphtheria and tetanus toxins, only a certain number of these respond freely and quickly to the stimulus of toxin injections, and are capable of producing antitoxins of the highest potency. It is probable that this variability among the individuals of the same species, in the response to stimulation by toxin, may extend to other forms of immunisation. It is quite conceivable that the dosage mentioned may have in this case been a sufficient stimulus to excite the mechanism necessary for antitoxin production in a highly responsive organism.

To ascertain whether the injection of heated filtrates had given rise to antitoxin production, the following preliminary test was carried out with the serum of horse "R," and controlled with the serum of normal horse "Z," the test toxin employed being Toxin No. 10. The toxin and serum were mixed and kept one hour at 35° C. It will be seen in Table XII. that the evidence is in favour of antitoxin production.

TABLE XII.

TEST OF SERUM OF HORSE "R."

Test Toxin No. 10. Toxin and Serum mixed and placed 1 hour at 35° C.

Rat.	Weight in grammes.	Volume of Toxin.	Volume of Serum.	Result.
1.	100	2 C.C.	1 C.C.	Died in 8 days.
2.	100	2 "	1 "	Lived.
3.	110	2 "	1 "	Lived.
CONTROL:—Serum of Normal Horse "Z."				
4.	100	2 C.C.	1 C.C.	Dead in 24 hours.
5.	100	2 "	1 "	Dead in 24 hours.
6.	110	2 "	1 "	Dead in 24 hours.

A test was carried out on one day with the serum of a number of horses. It will be seen from the Table XIII. that the only serum which showed marked antitoxic properties was the serum of horse "M," which had been immunised with toxins only.

TABLE XIII.

SERUM OF SEVERAL HORSES VARIOUSLY IMMUNISED.

Test Toxin No. 10; tests all carried out on one day.

Toxin and Serum mixed and kept 1 hour at 35° C

	Rat No.	Weight in grammes.	Vol. of Toxin.	Vol. of Serum.	Result.
Horse "M."	1.	95	2 C.C.	1'0 C.C.	Lived.
	2.	100	"	0'1 "	Lived.
	3.	100	"	0'02 "	Lived.
Horse "R.D."	4.	100	2 C.C.	1'0 C.C.	Lived.
	5.	90	"	0'1 "	Died 48 hours.
	6.	90	"	0'02 "	Died 24 hours.
Horse "M.B."	7.	90	2 C.C.	1'0 C.C.	Lived.
	8.	90	"	0'1 "	Died 48 hours.
	9.	90	"	0'02 "	Died 48 hours.
Horse "P.N."	10.	100	2 C.C.	1'0 C.C.	Died 48 hours.
	11.	95	"	0'1 "	Died 48 hours.
	12.	95	"	0'02 "	Died 24 hours

The test on the serum of "M." was repeated and controlled with the normal horse "N. H.," see Table XIV. The same Test Toxin No. 10 was used, and the toxin and serum were mixed and kept two hours at 35° C.

TABLE XIV.

TEST ON SERUM OF "M." REPEATED.

Serum of Normal Horse "N. H."

Rat No.	Weight in grammes.	Volume of Serum.	Volume of Toxin.	Result.
1.	100	1 C.C.	2 C.C.	Died 24 hours.
2.	90	0.5	2 "	Died 48 hours.
3.	95	0.1	2 "	Died 48 hours.
Serum of Horse "M."				
4.	100	1 C.C.	2 C.C.	Lived.
5.	100	0.1	2 "	Lived.
6.	95	0.02	2 "	Lived.
7.	90	0.01	2 "	Died 24 hours.

Reference has been made to the serum of the Horse "Pr." The results of a number of tests relating to the serum of this horse are shown in the accompanying Tables :—XV., XVI., XVII. and XVIII.

TABLE XV.

TESTING SERUM OF "Pr." (6. IV).

Mice of 15-20 grammes weight.

Toxin (Subcutaneously at least = 4 lethal doses; intraperitoneally = 10 lethal doses).

Serum and Toxin mixed and placed 1 hour in the incubator at 37° C.

Mixture injected subcutaneously.

Mouse.	Volume of Toxin.	Volume of Serum.	Result.
1.	'2 C.C.	'1 C.C.	Lived.
2.	'2 "	'1 "	Lived.
3.	'2 "	'2 "	Lived.
4.	'2 "	'2 "	Lived.

Mixture injected intraperitoneally.

5.	'2 "	'1 "	Lived.
6.	'2 "	'1 "	Lived.
7.	'2 "	'2 "	Died in 48 hours.
8.	'2 "	'2 "	Lived.

CONTROLS :—Same quantities of Toxin; serum of normal Horse "F."

Subcutaneous.

9.	'2 C.C.	'2 C.C.	Died 24 hours.
10.	'2 "	'2 "	Died 16 hours.

Intraperitoneal.

11.	'2 "	'2 "	Died 24 hours.
12.	'2 "	'2 "	Died in 10 hours.

TABLE XVI.

TESTING SERUM OF "Pr." (27, IV.) ON RATS.

Test Dose about 10 M. L. D.

Toxin and Serum mixed and kept 2 hours at 37° C., then injected subcutaneously.

Mouse.	Weight in grammes.	Volume of Toxin.	Volume of Serum.	Result.
1.	120	1'0 C.C.	'01 C.C.	Died 24 hours.
2.	120	"	'02 "	Lived.
3.	115	"	'05 "	Lived.
4.	115	"	'1 "	Lived.
5.	115	"	'3 "	Lived.
6.	110	"	'5 "	Lived.
7.	105	"	1'0 "	Lived.
8.	100	"	1'0 "	Died in 27 days.
CONTROLS :—Done with Serum of Normal Horse "F."				
9.	120	1 C.C.	2 C.C.	Died 3 days.
10.	105	"	2 "	Died 3 days.
11.	110	"	1 "	Died 24 hours.
12.	120	"	1 "	Very ill ; recovered.*

* The rats frequently bite the site of inoculation, and in certain cases, as the result of this, leakage may take place.

TABLE XVII.

TESTING SERUM OF "Pr." ON MICE.

The Serum injected subcutaneously at one side of the body, and 24 hours later the Toxin at the other side.

Test Toxin No. 10 about 4 to 6 M. L. D.

Only small mice available.

Mouse.	Weight in grammes.	Volume of Serum.	Volume of Toxin.	Result.
1.	8	'6 c.c.	'3 c.c.	Lived.
2.	10	'6 "	'3 "	Lived.
3.	10	'6 "	'3 "	Lived.
4.	10	'6 "	'4 "	Lived.
5.	10	'6 "	'4 "	Lived.
6.	10	'6 "	'4 "	Lived.

CONTROLS :—With Serum of "B." (old Diphtheria Horse).

7.	8	'6 c.c.	'3 c.c.	Very ill ; recovered.
8.	10	'6 "	'3 "	Died 4 days.
9.	10	'6 "	'3 "	Died 7 days.
10.	10	'6 "	'4 "	Died 48 hours.
11.	10	'6 "	'4 "	Died 48 hours.
12.	15	'6 "	'4 "	Very ill ; died 12 days.

TABLE XVIII.

TESTING SERUM OF "Pr." ON RATS (27, IV.).

Toxin No. 10.

Serum injected on one side of body ; Toxin (at least 10 M. L. D.) 24 hours later on the other side.

Rat.	Weight in grammes.	Both subcutaneous at opposite sides of abdomen.		Result.
		Serum.	Toxin, 24 hours after Serum.	
1.	95	1 C.C.	1.5 C.C.	No symptoms.
2.	95	"	"	No symptoms.
3.	100	"	"	No symptoms.
4.	105	"	"	No symptoms.
5.	115	"	"	No symptoms.
6.	120	"	"	No symptoms.
CONTROLS :—With Normal Serum of Horse "Z."				
7.	95	1 C.C.	1.5 C.C.	Died 24 hours.
8.	100	"	"	Died 48 hours.
9.	100	"	"	Died 24 hours.
10.	110	"	"	Died 48 hours.
11.	115	"	"	Ill ; recovered.
12.	120	"	"	Died 48 hours.

These tests appear to show :—

1. That the serum is capable of neutralising the toxin when mixed *in vitro*, as tested by subsequent subcutaneous or intraperitoneal injection into rats and mice.
2. That the serum injected into rats or mice, 24 hours before the toxin, is capable of conferring protection on these animals.
3. In the case of rats, 0.02 c.c. of serum, when mixed with the toxin, neutralises the toxic action of about 10 M. L. D., and 1 c.c. of serum protects against at least 10 M. L. D. injected at another part of the body 24 hours after the serum.

Kolle says that the serum of the normal horse has considerable antitoxic properties, but, as already stated, he was unable to obtain an increase beyond what he regarded as within the limits of normal variation.

If the serum of an animal normally contains antitoxin, we might anticipate on the basis of Ehrlich's theory that it would be possible by stimulating the organism to obtain an increase in the production of the antitoxin. In our experiments, for purposes of control, the sera of four normal horses were employed, but, in addition, the serum of five horses immunised by the ordinary method of obtaining anti-plague serum showed little or no increase in antitoxic power, and these may, therefore, be classed among the controls.

The evidence from these experiments seemed to be in favour of the possibility of obtaining a serum with antitoxic properties in excess of those found in normal serum.

Relation of Neutralisation of Toxin to Precipitin Reaction.—Certain experiments were carried out with the view of ascertaining whether the occurrence of the precipitin reaction of Kraus might not play some part in the plague toxin-antitoxin reaction.

The serum of certain of the horses gave a well marked precipitation with the Test Toxin No. 10. The serum of the horse "R D." gave 3 to 4 times the precipitum given by the serum of the horse "M." In the previous tests we have seen that whereas 1 c.c. of the serum of "R D." was required to neutralise 10 M. L. D. of Toxin 10, 0.02 c.c. of the serum of "M." produced the same effect. There is, therefore, no direct proportionality between the two properties of the serum.

The Protective Action of Anti-Plague Serum.—Though much sceptical criticism has been directed against anti-plague serum, it has been admitted by everyone who has seriously approached the subject that, however disappointing the results of its use on the human subject may be, it manifests definite protective properties when tested on animals. A typical test showing the effect on rats is shown in the annexed Table XIX.

It will be noted that death occurred in the case of the animals which received the largest dose of serum. The writer has observed similar results in the case of anti-streptococcic serum. Tavel, Krumbein, and Glücksmann also record similar results, and attribute them to the well-known phenomena of "diversion of the complement" easily demonstrated in test tube experiments on bacteriolysis.

A common experience is to find that animals treated with serum survive till about the tenth day, and then succumb to the disease. This is probably due to the fact that the immune serum has by that time been metabolised and excreted, and that some of the bacilli which have not been destroyed are able to re-infect the animal.

We have already seen that the antitoxic properties of the serum of the horse "MB." are not of a high order, and probably do not exceed the limits found in normal serum.

A number of tests were carried out to determine whether the serum possessed bacteriolytic properties. The methods employed and the results obtained are dealt with in the following section.

Testing Plague Serum for Bacteriolytic Properties.

A number of methods were employed to ascertain whether bacteriolysis could be demonstrated in the case of anti-plague serum. Neisser's (1904) technique was found to give the most consistent and accurate results.

Small test tubes of about $10 \times 1\frac{1}{2}$ c.m. are employed. Suitable dilutions of the immune serum are prepared in normal salt solutions, say, 1 : 10, 1 : 100, 1 : 1000, etc., and the quantity of serum to be tested is pipetted into each test tube of a series, and to this 0.5 c.c. of fresh normal serum is added to supply complement. Three drops of bouillon are introduced into the mixture, and the volume in each tube is brought up to 2 c.c. A measured quantity of a suitable dilution of the culture is

(140)

TABLE XIX.
TESTING SERUM OF HORSE "MB."

Rat No.	Weight.	Serum 24 hrs. before toxin.	Infection.	Result and Remarks.												
				Days	1	2	3	4	5	6	7	8	9	10		
																Survivors killed on 15th day after inoculation.
1.	215	'01	Shaving cutaneous				+									
2.	175	'05	"					+								
3.	195	'1	"							+						
4.	110	'3	"													
5.	120	'5	"													
6.	210	1'0	"													
7.	190	2'0	"													
8.	145	3'0	"													
9.	220	4'0	"													
10.	120	10'0	"									+				
Controls.																
1.	240	0	"				+									
2.	240	0	"							+						
3.	300	0	"				+									
4.	200	0	"				+									
5.	225	0	"						+							

Note.—"+" indicates death.

then introduced into each tube. The tubes are placed in the incubator at 37° C., and are examined at stated intervals, say in 3, 16, 24-48 hours, the same volume from each tube being pipetted on to an agar tube or Petri dish. The absence or diminution in the number of colonies on these tubes or plates after their incubation is, with certain reservations, regarded as the indication of bacteriolysis having taken place. The condition of the small test tubes, after their incubation for 24-48 hours, affords valuable information, as will be exemplified in the following test:

Test of the Serum of Horse "RD."—The arrangement in this experiment was as above. The bacterial dilution was prepared by emulsifying a 2 mgm. öse from a 24 hours culture on agar of a highly virulent plague bacillus in 10 c.c. of normal salt solution (85%). The bacilli were well distributed by shaking, and a 2 mgm. loopful of this dilution was introduced into each of the tubes containing the immune serum, fresh horse serum and broth. The agar tube planted at once from the control tube, which contained salt solution and bouillon but no serum, gave a fair number of colonies, which became confluent over the water of condensation. A measured quantity from each tube was planted on agar, after 3 and after 16 hours. The details and result of the experiment are shown in the Table XX.

TABLE XX.

Volume of Anti-plague Serum, Horse "R."		Volume of complement normal horse serum (16 hours old).	Condition in agar tubes, after 24 hours at 37° C.	Condition in tube containing immune serum and complement after 48 hours.
10	c.c.	0.5 c.c.	0	Growth in agglutinated colonies through the fluid.
.3	"	"	0	" "
.1	"	"	0	" "
.03	"	"	0	" "
.01	"	"	0	" "
.003	"	"	Colonies; few.	" "
.001	"	"	Colonies; few.	" "
.0003	"	"	Colonies; many.	Homogeneous growth.
.0001	"	"	Colonies; many.	" "
0	"	"	Uniform growth.	" "

The experiment shows, therefore, that while there was an apparent inhibition of growth, probably largely due to the agglutinating power of the serum which was 1 : 300 with a virulent culture, in no single tube had complete destruction of the bacilli taken place, since growth occurred in every one of the tubes containing the serum mixture.

A number of similar experiments, some of them carried out with the immune horse serum so fresh that the addition of complement was unnecessary, gave perfectly consistent results, *e.g.*, the fresh serum of horse "M B." was tested as above with a bacillus of low virulence, in full strength and in dilutions of 1-10, and 1-100, and 1-1000, and growth took place in all the tubes.

No matter what method was adopted, it was found impossible to obtain evidence of the serum possessing any bacteriolytic property, even when bacilli of low virulence were used.

Discussion as to the Nature of the Mechanism by which Anti-Plague Serum Exercises its Anti-Infectious Effect.

In considering the properties of anti-plague serum we have seen that an antitoxic action can play little part in the protective influence exerted by this serum. It is conceivable that the plague bacillus produces and throws off a large number of toxic particles or receptors under the stimulus of the antagonistic influences which it encounters when introduced into the animal body. If this be the case, it seems reasonable to assume that the bacilli, even when grown in artificial media, possess, though in smaller numbers, those toxic particles which are liable to be set free, especially on the death or during the disintegration of the microbes.

In dealing with the literature of plague toxin reference has been made to several methods of immunisation which are based on the common assumption that certain toxic or immunising substances are produced in a concentrated form in the animal body from which they are recoverable. Thus Terni and Bandi, Hueppe and Kikuchi used peritoneal exudates, and Klein the necrotic areas resulting from plague infection in different organs, as their immunising agent.

The first work in this direction was done by Denys and Van de Velde (1895) who showed that a substance "leucocidine," highly toxic for leucocytes, could be obtained in the pleural exudates resulting from the injection of staphylococci into the pleural cavity of the rabbit, and that the serum of rabbits immunised against staphylococci contained a substance "anti-leucocidine," capable of neutralising the toxic action of leucocidine. The leucocidine could be demonstrated to be present in cultures though not so abundantly as in the exudates.

The whole subject of the toxic and immunising action of the body fluids of infected animals has been re-opened by the investigations of Bail and his co-workers. This work embraces a theory of the method of attack on the animal body by certain groups of bacilli, and of the nature of the defence by means of which immunity is attained. Bail's views may be briefly stated as follows:—Certain organisms obtain a footing in the animal body by means of substances which they throw off, and to which Bail gives the name of "aggressins." These are demonstrable in the bacterial-free body-fluids, exudates, etc., of the infected animal.

The aggressive property of such fluids can be shown in the following manner. A non-lethal dose of the bacillus becomes lethal when mixed with its specific aggressin. The presence of aggressin, along with a lethal dose, causes the occurrence of a disease of a severer and more acute type, and one in which the pathological lesions are of a more pronounced character.

It is, in the case of the strictly parasitic bacilli, such as anthrax, fowl-cholera and plague, where a single virulent organism is probably sufficient to cause infection, that the aggressin action is most marked. The living bacillus can, without losing its vitality, secrete or throw off particles which prevent its destruction by phagocytes, and enable it, at the same time, to multiply and produce its pathogenic effects.

The immunity obtained by the injection into the animal body of natural aggressins is attributed by Bail to the production of anti-aggressins.

Wassermann and Citron (1905) hold, that aggressins can be obtained outside the animal body by shaking bacteria in serum or in distilled water. The fluids obtained by these means, after removal of the living germs, are capable of aiding the infective process. Their view is, that the aggressins, whether obtained from the animal body or by this method,

act by combining with the lysin of the serum, and thus interfere with the bacteriolytic process.

Such artificial aggressins must be regarded as closely related to certain bacterial toxins. One need only recall here, that the method employed by Wassermann and Citron is practically identical with Besredka's method for obtaining plague toxin.

The powerful immunising action of the natural aggressins, compared with the weak immunising action of these artificial aggressins, is used by Bail as an argument in favour of the essential difference in the nature of the two groups of bodies.

It is impossible here to enter into a full discussion of the relation of the aggressins to the "free receptors" of Neisser and Shiga (1903), and to the substance, demonstrated by Pfeiffer and Friedberger (1905), in serum, after its treatment with emulsions of typhoid and cholera organisms.

The intimate relation which these subjects bear to plague immunity is emphasised by certain work carried out in the Laboratory here, by Captain Douglas, I.M.S. (1906), recorded in a paper read at the Pathological Society of London, in which he showed that, contrary to the view taken by the German and English Plague Commissions, the supernatant fluid of Haffkine's Prophylactic Fluid has powerful immunising properties, as had been previously maintained by Haffkine. Where the prophylactic fluid was prepared by emulsifying the bacillus from agar in normal salt solution, the supernatant fluid, in this case also, was demonstrated to possess an immunising property. The writer has shown that the injection of the supernatant fluid in the horse gives rise to the production of a small amount of antitoxin. It is difficult to reconcile with these results of Douglas the statement made by Bail and Weil (1906), that attempts to immunise with watery extracts against the true parasites prove absolutely futile, since the *B. pestis* has been classified by them in this group.

Neisser and Shiga in the case of the dysentery bacillus of Shiga and the typhoid bacillus, obtained analogous results which they attributed to the presence of free receptors in the filtrates from the autolysed bacilli. In that case bacteriolytic sera were obtained ; in the case of plague we have shown that no matter whether the two fluids employed by Douglas and the writer or the living bacillus are used in the treatment of the

animals, the serum obtained does not owe its anti-microbial properties to bacteriolysis. The explanation offered by Wassermann and Citron, of the action of the watery extracts in hindering the anti-bacterial action of a serum, viz., that the suspended bacillary particles intervene in and prevent the bacteriolytic process, cannot, in this case, be regarded as altogether appropriate, unless the preparation of the bacilli for phagocytosis by the serum is regarded as a stage in an incomplete bacteriolysis. Neither can the immunity in the case mentioned be reasonably regarded as the result of the production of anti-aggressins, even if we assume that the natural and artificial aggressins are identical. Bail states that, at least in the case of typhoid and cholera, heating to 60° C., severely damages the aggressin, whereas in this case a temperature of 70° C. was employed. The conditions, therefore, for anti-aggressin production were not fulfilled in these experiments. It is, however, conceivable that an aggressin modified by heating might still be capable of stimulating to the formation of an anti-aggressin, just as a modified toxin or toxoid is capable of stimulating to antitoxin production.

The questions which are of great interest in relation to immunity generally, and especially to plague immunity, viz., whether the natural and artificial aggressins are the same or different bodies, their relation to toxins, and whether there exists an aggressin immunity *sui generis*, must be regarded as subjects still *sub judice*.

It has been shown in the preceding part of this paper that the protection conferred by anti-plague serum is little, if at all, dependent on antitoxic action. Nor does the humoral view of immunity which has been applied by many writers, among whom may be mentioned Kolle and Martini (1902), give a satisfactory explanation of its action since, as has been shown, no complete bacteriolysis *in vitro* can be demonstrated.

We must now, therefore, consider the part played by the serum in relation to phagocytosis, the importance of which as a factor in immunity is now universally admitted. Metchnikoff (1901), in reviewing the subject of the anti-infective or protective sera, says that the action may be direct on the micro-organism, and that it may be microbicidal, properly so-called, agglutinative, or fixative. In addition to these properties, and acting along with them, he upholds the view that these sera have a stimulating effect on the leucocytes.

The subject with especial relation to plague has been studied among others by Denys and Tartakowsky (1900), who attributed the anti-microbial action to the influence exerted by the serum on the process of phagocytosis.

Bordet and Gengou (1901) demonstrated the presence of substance sensibilisatrice in anti-plague serum by their method which is based on the observation that only sensitised bacilli are capable of removing alexin or complement from a fresh serum. The hæmolysis of sensitised red blood corpuscles is used as the test for free complement.

Employing this method, they showed that plague bacilli, which had been in contact with heated immune serum, removed the complement from fresh serum, and must, therefore, have been sensitised. These authors point out that certain micro-organisms, such as the *B. pestis*, are capable in this way of absorbing complement without undergoing any morphological changes, though the possibility of a physiological change having been effected is not excluded.

Markl (1903) made a very thorough investigation into the mechanism of the action of anti-plague serum. He found that all the phenomena could not be explained on the assumption that the serum had a bacteriolytic action, since bacteriolysis takes place only in the case of bacilli of low virulence. When he submitted plague bacilli *in vitro* to the action of an immune serum which had been reactivated by fresh normal serum, and made microscopical preparations at intervals of time, he found that the bacilli showed no marked changes such as would indicate that they had suffered any harm. On the other hand, if, in place of the normal serum, he used peritoneal lymph containing leucocytes, he found the leucocytes packed with bacilli which lost their normal staining reaction, and, at the end of 12 hours, in the case of bacilli of moderate virulence, the bacilli were represented only by dust-like detritus. Markl holds that, in the case where peritoneal lymph, rich in leucocytes, is used to reactivate the immune serum, not only is there great destruction of the bacilli within the leucocytes, but there occurs extra-cellular destruction of the bacilli which is inversely proportional to the virulence of the bacillus.

When a highly virulent plague culture is injected intraperitoneally the multiplication of the bacilli can be followed. There is very little exudate, in which only a few leucocytes are present, and there is a rapid increase in the number of bacilli followed by death in 24 hours.

When anti-plague serum has been injected into the animal 24 hours before the bacillus, there is observed, during the first hour, an increase in mononuclear leucocytes, around which the bacilli are clumped, some of them being englobed by the mononuclear leucocytes. After the first hour the polynuclear leucocytes multiply rapidly and englobe the bacilli, so that in three hours no free bacilli, and in six hours no bacilli either intra or extra-cellular, are observable. The exudate now consists entirely of polynuclear leucocytes. The animal in this case recovers.

When the number of bacilli is so great that the resistance conferred by the serum is insufficient to protect the animal, the bacilli which remain undevoured by the phagocytes continue to multiply till the death of the animal results.

The bacilli lying free and those within the mononuclear leucocytes retain their normal morphology and staining reactions, whereas, those within the polynuclear leucocytes tend to stain badly and to assume coccal-like forms.

In regard to the nature of the action of the serum in relation to phagocytosis, Markl says:—"To determine whether the injection of the immune serum has an influence on the cells of the body, or whether the bacilli are directly influenced, the following experiment was performed. A suspension of plague bacilli in normal salt solution was added to inactivated immune serum, and the mixture was placed three hours in the incubator. After centrifugalisation the clear fluid was pipetted from the sediment, which, after being several times washed with salt solution, was injected intraperitoneally into rats. These bacilli thus acted on by the immune serum behaved in the animal body exactly like bacilli which had been injected along with free immune serum. They were englobed by the phagocytes.

The phagocytosis occurred so rapidly and was so extensive that no doubt remains that the immune serum has a direct influence on the bacilli, entering into union with them and conferring on them a positive chemiotaxis." *

He concludes that in the case where bacilli of low virulence are injected extracellular bacteriolysis plays an important part, whereas,

* A discussion of the relation of Markl's work to the views of Douglas and Wright is avoided in this paper as of too controversial a nature.

where highly virulent bacilli are injected, phagocytosis is the only agency at work in their destruction.

The writer's experience and results are largely in agreement with Markl's, but, as already mentioned, in dealing with the subject of bacteriolysis *in vitro*, evidence of complete destruction of the bacilli, even of low virulence, could not be obtained. The results observed by Markl when the bacilli were injected into the peritoneum can also be obtained in the subcutaneous tissue. The writer has found that when a bacillus of high virulence is injected into the subcutaneous tissue of the abdomen, or where infection is caused by shaving the skin and rubbing the culture on the shaved area, *i.e.*, by the cutaneous method, an enormous multiplication of the bacilli takes place within the lymph spaces, which become distended with lymph in the vicinity of the point of inoculation. Microscopical preparations from this region show a complete, or almost complete, absence of leucocytes. On the other hand, if the animal has been immunised by an injection of anti-plague serum, given say 24 hours before the injection of the bacilli, instead of the diffuse indefinite swelling, or absence of any swelling, visible to the naked eye found in the unprepared rat, a well marked generally sharply circumscribed swelling is observed, and microscopical examination shows in it the presence of an enormous number of polymorphonuclear leucocytes, many of which are packed with bacilli. Animals in which this condition is observed either recover or die at a much later period than the controls.

Summary.

Toxins can be demonstrated in the filtrates of bouillon cultures of the *B. pestis*. These toxic substances are probably set free by the disintegration of the bacillus through autolysis, but, since Albrecht and Ghon and Markl were able to demonstrate their presence in young cultures, the possibility is not excluded that they are toxins in the narrower sense, *i.e.*, secretory products of the bacillus. The toxin is much more toxic for the rat and mouse than for the rabbit and guinea-pig. The toxicity for mice is destroyed by heating to 70° C. for 30 minutes.

Under certain conditions, the injection of toxin stimulates the animal body to the formation of antitoxin, and the experiments recorded here

appear to show that this antitoxin is capable of neutralising the toxin, either when mixed with it or when the serum is injected twenty-four hours before the toxin, and when the two substances are injected at different parts of the body.

It seems doubtful whether it will be possible to maintain a hard and fast line of demarcation between the toxins proper and the endotoxins. The injections of emulsions of bacilli from agar, as well as of the toxic filtrates from broth cultures, may, in certain cases, stimulate the animal organism to the production of an antitoxic serum as was shown in the Laboratory here, by Todd (1904), in the case of the *B. dysenteriae* of Shiga. The view that it is a general biological law, as stated by Kolle, that the injection of endotoxins into the animal body does not stimulate to antitoxin production, cannot be unreservedly accepted, but must be subjected to further investigation.

Whether the toxin and antitoxin in the case of plague differs or not essentially from those obtained in the case of diphtheria and tetanus, it must be admitted that it is impossible to obtain them in anything like the same degree of concentration.

The neutralisation of toxin by the antitoxin *in vitro* is not due to the occurrence of a precipitin reaction. The serum of a horse giving a much greater precipitum with a particular toxin may possess a much lower antitoxic value than the serum of a horse which gives a much smaller precipitum with the same toxin. The fact that the neutralisation also takes place *in vivo* is against the view that a precipitin reaction plays a part in the phenomenon, for Rostoski, Michaelis and Oppenheimer (1902), showed that this reaction never takes place in the blood of the living animal.

So far as has been demonstrated experimentally, antitoxic and bacteriolytic actions play little part in the protection afforded by the anti-plague serum. The action of the serum can probably be best interpreted as follows:—The serum acts, not by any influence on the cells of the animal body, but directly on the bacilli, which, after being submitted to its action, no longer repel but rather attract the leucocytes, by which the microbes are then englobed and digested. This was first shown in the case of the *B. pestis* by Markl.

The change in the microbe has been expressed by stating that the immune serum converts the negative chemiotaxis of the bacillus into

a positive chemiotaxis. The substance producing the change has been variously designated as *fixateur*, *substance sensibilisatrice*, *opsonin* and *incitor*.

It is still an open question whether we are here dealing with a stage in an incomplete bacteriolysis in which the amboceptor acts as the intermediary between the bacillus and the leucocyte. The view is one which can be maintained with much reason. Certain organisms, such as the *Cholera vibrio*, when acted on by an immune serum containing amboceptor and complement, undergo bacteriolysis, which can be seen under the microscope, or estimated by the absence of growth. Other organisms, such as the *B. pestis*, are acted on by an immune serum, but in this case the solution or death of the bacillus does not take place outside the cells. That the organism has, however, suffered change is shown by the altered behaviour of the leucocytes to which we have just referred.

Whether the bacillus undergoes an extra-cellular bacteriolysis, or is digested after being phagocytosed, it must not be overlooked, that in both cases it is highly probable that the ultimate protection of the animal is effected by the phagocytes, which, in the former case, take up the toxic particles set free by bacteriolysis, in the latter, take up and digest the bacillus and retain the toxic particles liberated during the process.

When the extra-cellular or intra-cellular bacteriolysis is carried on with too great energy so that the leucocytes are not strong or numerous enough to deal with the toxic products, the death of the animal results.

The question as to whether anti-plague serum owes any of its protective power to the "stimulines" of Metchnikoff and the "anti-aggressins" of Bail must be decided by further investigations.

Conclusions.

1. That toxins can be obtained from broth cultures of the plague bacillus.
2. That these toxins are probably endotoxins, as the toxicity of the filtrate increases with the age of the culture, and becomes considerable only after many of the bacilli have been autolysed.

3. That the injection of large doses of these toxic filtrates is capable of stimulating the animal body to the formation of an antitoxin.

4. That the neutralisation of the toxin by antitoxin is not merely due to a precipitin reaction for (a) it occurs *in vivo* where no precipitin reaction takes place, and (b) a serum with little or no antitoxic properties may be capable of producing a marked precipitin reaction and *vice versa*.

5. That neither antitoxic nor bacteriolytic actions play much part in the protection afforded by an anti-plague serum.

6. That the anti-microbial action of an anti-plague serum is, as was first shown by Markl, almost, if not entirely, due to a direct action of the serum on the microbe, by means of which its negative chemiotaxis is converted into a positive chemiotaxis, so that it no longer repels but rather attracts the phagocyte. The substance in the serum producing this action is thermostable, and has been variously named fixateur, substance sensibilisatrice and incitor.

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EXPERIMENTAL STUDY OF THE IMMUNITY
AGAINST BACILLUS PYOCYANEUS.

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Experimental Study of the Immunity against *Bacillus Pyocyaneus*.

Introduction.

SINCE Géssard⁽¹⁾ in 1882 isolated pure cultures of *bacillus pyocyaneus* for the first time, this remarkable microbe has been the subject of a great amount of investigation. A special interest in its pathogenic properties was aroused by the publication in 1889 of Charrin's monograph on "la maladie pyocyannique." Charrin⁽²⁾ noted that pigeons, guinea-pigs, rabbits and frogs were susceptible, and in particular that the rabbit exhibited certain characteristic symptoms. In this animal he witnessed several types of the pyocyanic disease, viz :—

1. Superacute type, fatal in 24 hours.
2. Acute type, fatal in 1 - 2 days.
3. Subacute type, fatal in 1 - 3 weeks.
4. Chronic type, fatal in several months.

In the chronic cases, after an incubation period lasting from 15 days to two months, Charrin saw the onset of paralysis affecting one or both hind limbs, and occasionally spreading to the anterior extremities. The paralysis was of a spastic type, and death was preceded by a condition of marasmus. Among the anatomical changes, Charrin observed enteritis, nephritis, and in one case amyloid infiltration.

By growing *bacillus pyocyaneus* in bouillon for a week, and then filtering the culture through porcelain, Charrin found that, in large doses (60-80 c.c.), the rabbit developed symptoms of disease.

In 1896 appeared the important research of Wassermann⁽³⁾, who demonstrated that the guinea-pig is the most sensitive animal to infection by the pyocyanic bacillus. The intraperitoneal inoculation of $\frac{1}{10}$ of a platinum loop of a culture was sufficient to cause death in 24 hours.

Wassermann also made special inquiry into the existence of a pyocyanic toxin, which he obtained by growing cultures of the bacillus for about six weeks, at the end of which time he destroyed the vitality of the culture by means of toluol. Such toluolised cultures were highly toxic, killing guinea-pigs rapidly in a dose of .5 c.c. Even with young toluolised cultures, he obtained evidence of toxic effect, and, as the bodies of pyocyanic bacilli killed with chloroform were much less toxic, he concluded that in the broth there must be a specific pyocyanic toxin. The toxin was not entirely robbed of its toxic properties by heating to 115° C.

In his immunisation experiments Wassermann found great differences, according as the animals were injected with the pyocyanic toxin or with the bacilli themselves. In the former case the serum protected against both the toxin and the culture, whereas the serum of animals, immunised with bacilli, protected only against the bacilli, but was unable to neutralise the lethal effect of the toxin. In most cases the immunised animals (guinea-pigs) could withstand many times the lethal dose of the living cultures, but only small multiples of the lethal toxin dose. As had been shown by Pfeiffer and Kolle in the case of cholera and typhoid, it was not possible to raise the immunity to an unlimited degree.

The nature of the immunity induced was also studied by Wassermann, and although he was unable to demonstrate any bactericidal powers in the immune serum *in vitro*, he found that *in vivo* the pyocyanic bacillus is destroyed in the peritoneal fluid, the destruction being accomplished without the agency of leucocytes. He considered that the process was similar to that which obtains in the case of typhoid and cholera. To the sera of animals immunised with increasing doses of pyocyanic toxin he ascribed true antitoxic properties.

Working along the same lines as Wassermann, Ghéorghiewsky (⁴) obtained divergent results, in so far that he found the pyocyanic bacillus to be taken up by the phagocytes in the peritoneal cavity, and destroyed in their interior.

When a non-lethal dose of bacillus pyocyaneus was introduced into the peritoneal cavity of a normal guinea-pig, there was a definite leucocytosis at the end of two to three hours, and within six hours all the injected bacilli were engulfed by the leucocytes, in the interior of which

they were destroyed. When a similar experiment was performed on a well-vaccinated guinea-pig, the intra-cellular destruction of the bacilli was quicker and more perfect. Ghéorghiewsky thus attributes the immunity against bacillus pyocyaneus to phagocytosis, and not to a destruction of the bacilli by the serum. He was unable to demonstrate any bactericidal action in the serum either *in vitro* or *in vivo*. Incidentally Ghéorghiewsky also noted a peculiarity of the immune serum which had been observed by Charrin, viz., that it prevents the bacillus from forming pyocyanin.

P. Müller⁽⁵⁾ was also unable to demonstrate any bactericidal powers in pyocyanic immune serum *in vitro* in aerobic conditions of the experiment. In anaerobic conditions, however, he considered that the bactericidal action was very marked, and that it could be inhibited by heating the immune serum to 55° C. The subsequent addition of a normal serum to the inactive heated serum restored the bactericidal power.

Margarethe Breymann⁽⁶⁾ made a special investigation into the question of the toxicity of filtrates of pyocyanic cultures. The organism grown in bouillon for periods varying from 7 - 50 days, was passed through the Chamberland filter, the filtrate being inoculated into mice, guinea-pigs and rabbits. She found that very large doses (100 c.c.) could occasion death of rabbits, with great emaciation, quantities less than this being practically without effect. Mice and guinea-pigs withstood respectively doses of 1 c.c. and 40 c.c. of the filtrate. On concentrating the filtrates over sulphuric acid, they were found to be non-toxic, even in quantities representing 100 c.c. of the original filtrate. The cause of this loss of toxicity was probably due to the disappearance of volatile bodies—the toxic action of which had been already maintained by Charrin⁽⁷⁾.

MacIntyre⁽⁸⁾ grew bacillus pyocyaneus in large quantities on agar, and after 14 days he precipitated with alcohol, collected the precipitate on a hard filter, and extracted with alcohol and ether. He found that 5 mgr. of the germ substance extracted in this way killed guinea-pigs, after intra-peritoneal injection, in 12 hours. The amount injected was about 1-50,000th of the body weight of the animal. Injected subcutaneously, one part of cell substance to 10,000 parts of body weight produced no permanent ill effects.

Heating to 120° C. in the autoclave did not rob the toxic substance of much of its toxicity. MacIntyre failed to produce immunity to intra-peritoneal injection by a previous series of subcutaneous inoculations.

In his most recent publication on the subject of pyocyanic immunity, Wassermann (9) still maintains the distinction between the antitoxic and bactericidal properties of the immune serum; although he admits that the antitoxin does not, as in the case of other antitoxins, follow the law of multiples. He explains this by assuming that the pyocyanic "toxin," in addition to the true exotoxin, contains endotoxin which has dissolved out of the bodies of the bacilli. The bactericidal action he still holds to be similar to that which obtains in the case of cholera, although the extra-cellular destruction of the *B. pyocyaneus* is slow compared with that of the *vibrio cholerae*.

Experimental.

Four races of *bacillus pyocyaneus* were used in the following experiments, and they were isolated from the throat, an ulcer of the leg, from the blood, and from the intestine respectively, of human beings. In all cases the cultures were highly pathogenic for guinea-pigs, and less so for rabbits.

When freshly isolated, one loopful of an agar culture injected into the peritoneum of a guinea-pig killed the animal acutely within 12 hours, the autopsy revealing intense peritonitis with great desquamation of endothelium, and accumulation of leucocytes in the thick fibrinous exudate. Minute hæmorrhages were numerous on the mesentery. The liver and kidney, on microscopic section, showed evidences of severe cloudy swelling. Cultivations from peritoneal exudate, liver, and heart blood, showed that the bacillus had become generalised over the body, the condition being really a pyocyanic septicæmia.

Cultures from the heart blood were inoculated into large flasks containing 3-6 litres of 1 % peptonised bouillon, and grown for periods varying from two days to three months. Within twenty-four hours a fine bacterial film appears on the surface, and within three or four days it has become converted into a thick membrane. The flasks were then thoroughly shaken, so as to dislodge the membrane to the bottom. As soon as a new membrane appeared, the process was repeated. When the flasks were kept untouched in the incubator for three to four weeks,

the general colour of the medium was a yellowish-brown. On shaking violently, this was instantly converted into a bright grass green colour from the action of the oxygen of the air, and the formation of pyocyanin and fluorescent green. In the course of time the consistence of the medium alters, so that, instead of the limpid clear broth, a thick stringy mucinous-like material occupies the whole flask.

Filtration Experiments.

Cultures of the bacillus in broth were taken at various times and passed through the Berkefeld filter. With young cultures up to 7 days this was easy, but when the mucinous cultures had to be dealt with, filtration was found to be extremely difficult, even the most open-pored Berkefeld filters becoming rapidly clogged up.

Filtrate from 2 day old broth culture.

Animal.	Quantity inoculated.	Result.
Guinea-pig.	5 c.c. intraperitoneal.	No effect.
"	10 c.c. "	No effect.
"	20 c.c. "	Slight loss of weight.
"	40 c.c. "	Loss of weight—recovery.
Rabbit.	5 c.c. intravenous.	No effect.
"	10 c.c. intraperitoneal.	No effect.
"	20 c.c. "	Slight loss of weight.
"	50 c.c. "	Loss of weight—recovery.

Filtrate from 7 day old culture.

Animal.	Quantity inoculated.	Result.
Guinea-pig.	10 c.c. intraperitoneal.	No effect.
"	30 c.c. "	Slight loss of weight.
Rabbit.	20 c.c. "	No effect.
"	40 c.c. "	No effect.

Filtrate from 30 day old culture.

Animal.	Quantity inoculated.	Result.
Guinea-pig.	20 c.c. intraperitoneal.	No effect.
Rabbit.	20 c.c. "	No effect.
"	40 c.c. "	Loss of weight—recovery.

Filtrate from 60 day old culture.

Animal.	Quantity inoculated.	Result.
Guinea-pig.	30 c.c. intraperitoneal.	Loss of weight—recovery.
Rabbit.	40 c.c. "	Loss of weight—recovery.

From these experiments, it is manifest that even large doses of filtrates of cultures of various ages fail to produce lethal effects. It was considered that increase in virulence of the cultures might lead to increased toxin production, and to this end the bacillus was passed through several series of guinea-pigs and rabbits, cultures being ultimately made from the last members of the series. Filtrates of these cultures were found to be incapable of producing lethal effects in doses up to 40 c.c. for guinea-pigs and 50 c.c. for rabbits. Wassermann has suggested, that although the toxin is largely a secretory product of the bacillus pyocyaneus, it may be kept back in the mucinous material, and in this way be prevented from passing the filter. This, however, does not apply to young cultures up to the first seven days, and here, as the experiments show, a soluble toxine, if such exist, is present only in very minute quantities.

Action of dead cultures of B. Pyocyaneus.

Dead cultures prepared in various ways were used in a number of experiments. Following Wassermann, the cultures were grown on bouillon or on agar. The bouillon cultures, after 2, 7, 21, 31 and 60 days,

were covered with a thick layer of toluol, and were then repeatedly shaken until complete sterility was obtained.

Agar cultures were grown *en masse* in Roux's bottles, the bacterial growth being subsequently washed off in a small quantity of normal saline solution. The thick emulsion obtained in this way was then removed by a pipette to a sterile stoppered bottle, and killed by toluol or chloroform.

Cultures in broth or on agar were also killed by heat at 65° C. and at 100° C., the toxicity being afterwards tested.

Effect of Toluolised Bouillon Cultures.

Animal.	Quantity inoculated.		Result.
Guinea-pig.	1 c.c.	2 day culture, intraperitoneal.	Dead 12 hours.
"	0.5 c.c.	" "	Dead 24 hours.
"	0.2 c.c.	" "	Very ill—recovered.
Guinea-pig.	1 c.c.	7 day culture, intraperitoneal.	Dead 12 hours.
"	0.5 c.c.	" "	Dead 24 hours.
"	0.2 c.c.	" "	Ill—recovered.
Guinea-pig.	1 c.c.	21 day culture, intraperitoneal.	Dead 8 hours.
"	0.5 c.c.	" "	Dead 12 hours.
"	0.2 c.c.	" "	Dead 3 days.
"	0.1 c.c.	" "	Ill—recovered.
"	0.05 c.c.	" "	Ill—recovered.
Guinea-pig.	1 c.c.	31 day culture, intraperitoneal.	Dead 8 hours.
"	0.5 c.c.	" "	Dead 24 hours.
"	0.2 c.c.	" "	Dead 4 days.
"	0.1 c.c.	" "	Very ill—recovered.
Guinea-pig.	1 c.c.	60 day culture, intraperitoneal.	Dead 24 hours.
"	0.5 c.c.	" "	Dead 6 days.
"	0.2 c.c.	" "	Ill—recovered.

From these results it is manifest that Wassermann's so called "toxin" can produce, in doses of .5 - 1.0 c.c. acute lethal effects. Post-mortem, the main changes are witnessed in the peritoneum, which presents evidences of acute inflammation, and in the liver and kidney, which are inflamed, and show acute cloudy swelling.

Effect of Broth Cultures killed at 65° C.

Animal.	Quantity inoculated.	Result.
Guinea-pig.	1 c.c. 7 day culture, intraperitoneal.	Dead 12 hours.
"	0.5 c.c. 7 " "	Ill—recovered.
"	1 c.c. 21 day culture, intraperitoneal.	Dead 12 hours.
"	0.5 c.c. " "	Dead 2 days.
"	1 c.c. 31 day culture, intraperitoneal.	Dead 24 hours.
"	0.5 c.c. " "	Ill—recovered.
"	1 c.c. 60 day culture, intraperitoneal.	Dead in 8 days.
"	0.5 c.c. " "	Loss of weight.

Effect of Bouillon Cultures exposed at 100° C. for ½ hour.

Animal.	Quantity inoculated.	Result.
Guinea-pig.	2 c.c. 7 day culture, intraperitoneal.	Ill—recovered.
"	1 c.c. " "	Ill—recovered.
"	2 c.c. 21 day culture, intraperitoneal.	Dead 4 days.
"	1 c.c. " "	Ill—recovered.
"	2 c.c. 31 day culture, intraperitoneal.	Ill—recovered.
"	1 c.c. " "	No effect.

Effect of Emulsions from 24 hour agar cultures killed by Toluol.

(A 24 hour culture, which covered the whole surface of a Roux's bottle, was emulsionised in 20 c.c. saline solution—the emulsion being then sterilised with toluol.)

Animal.	Quantity injected.	Result.
Guinea-pig.	1 c.c. emulsion.	Dead 24 hours.
"	0.5 c.c. " "	No symptoms.

Effect of 24 hour agar cultures killed by chloroform.

A 24 hour culture which covered the whole surface of a Roux's bottle was exposed to chloroform vapour, the bottle being well closed by an indiarubber bung.

Before the action of the chloroform, 0.1 of a loopful of the living

culture killed a guinea-pig in 12 hours. 10 loopfuls of the chloroformed culture produced no symptoms in another guinea-pig.

1 c.c. of a very thick emulsion of a chloroformed culture produced death in 4 days.

From these experiments it is manifest that toluolised bouillon cultures are lethal in doses of .5 - 1 c.c. The bacillary bodies themselves, killed by toluol or chloroform, are much less toxic. The toxicity of toluolised broth cultures may be largely due to poisons which have slowly diffused out of the bacillary bodies. It is also possible that it is due to secondary products produced by the fermentation of the medium (bouillon) in which the bacillus has been growing. The absence of toxic action in young filtrates is in favour of the latter view, and against the opinion of Wassermann, that the so-called "pyocyanic toxin" is a true exotoxin.

Effects of Autolysed Cultures.

Autolysed cultures were made according to the methods of Conradi⁽¹⁰⁾ and Neisser⁽¹¹⁾. Following the first of these methods, bacillus pyocyaneus was grown in Roux's bottles at 37° C. for 20 hours. At the end of this time the culture mass was washed into a saline solution and allowed to digest in this for 24 hours, after which time the fluid was passed through the Berkefeld filter.

In the case of autolysates made by Neisser's method, large agar cultures were suspended in saline solution, heated to 60° C. for 1 hour, and then placed in the incubator for 2 days to digest. At the end of this time the fluids were passed through the filter.

Animal.	Amount inoculated.			Result.
Rabbit.	5 c.c.	autolysate (Conradi)	intraperitoneal.	No effect.
"	10 c.c.	"	"	No effect.
"	15 c.c.	"	"	No effect.
"	10 c.c.	"	intravenous.	No effect.
"	10 c.c.	(Neisser)	intravenous.	No effect.
"	15 c.c.	"	intraperitoneal.	No effect.
"	20 c.c.	"	"	Death in 8 days.

Considering the large quantity of bacilli employed in the preparation, these autolysates appear to be practically non-toxic in doses up to 15 c.c. A lethal effect was first seen in doses of 20 c.c.

*Virulence of B. Pyocyaneus obtained direct from man.—Conservation
and Exaltation of Virulence.*

The virulence of the pyocyanic bacillus obtained from man varies. In the case of the races obtained from the leg ulcer and from the intestine, the virulence of the first subcultures were such that 1 loopful (2 mgr.) injected intraperitoneal caused an acute lethal pyocyanic sepsis in the guinea-pig within 12 hours. With cultures from the throat and from the blood, the virulence was such that .25 loopful killed guinea-pigs acutely. The virulence was well maintained, even for months, in cultures grown on agar stabs at 37° C. for 24 hours, and subsequently at 20° C.

From each of the four races used in the experiments, passages were made through both rabbits and guinea-pigs to determine whether and to what extent exaltation is possible. After the death of the animal the bacillus was recovered from the heart blood, grown on agar for 24 hours, and inoculated into the peritoneum of the next animal in series.

No.		Dose.		Result.
Rabbit	1.	2 loops culture, intraperitoneal.		Death 12 hours.
"	2.	1	" "	Death 3 days.
"	3.	1 loop culture from Rabbit	1, intraperitoneal.	Death 12 hours.
"	4.	.5	" " 1, "	Death 7 days.
"	5.	1 loop culture from Rabbit	2, intraperitoneal.	Death 12 hours.
"	6.	.5	" " 2, "	Death 12 hours.
"	7.	.4	" " 2, "	Death 2 days.
"	8.	.4 loop culture from Rabbit	5, intraperitoneal.	Death 2 days.
"	9.	.3	" " 5, "	Death 5 days.
"	10.	.3 loop culture from Rabbit	8, intraperitoneal.	Death 3 days.
"	11.	.25	" " 8, "	Recovery.
"	12.	.3 loop culture from Rabbit	10, intraperitoneal.	Death in 2 days.
"	13.	.25	" " 10, "	Death in 5 days.
"	14.	.25 loop culture from Rabbit	12, intraperitoneal.	Death in 8 days.
"	15.	.20	" " 12, "	Recovery.

In neither of the four series in rabbits did less than .3 loopful kill acutely irrespective of the number of passages.

Guinea-pigs (intraperitoneal inoculation).

No.	Dose.				Result.
1.	1 loop culture.				Dead 8 hours.
2.	.5	"			Dead 12 hours.
3.	.3	"			Dead 24 hours.
4.	.1	"			Dead 4 days.
5.	.3 loop from guinea-pig 3.				Dead 8 hours.
6.	.2	"	"	3.	Dead 12 hours.
7.	.1	"	"	3.	Dead 24 hours.
8.	.1	"	"	7.	Dead 12 hours.
9.	.05	"	"	7.	Dead 4 days.
10.	.1	"	"	8.	Dead 12 hours.
11.	.05	"	"	8.	Dead 24 hours.
12.	.03	"	"	8.	Ill—recovered.
13.	.05	"	"	11.	Dead 24 hours.
14.	.04	"	"	11.	Dead 8 days.
15.	.03	"	"	11.	Ill—recovered.
16.	.04	"	"	14.	Ill—recovered.
17.	.04	"	"	14.	Ill—recovered.

Although the virulence of the cultures was considerably greater than has been reported by most authors, it was found impossible to exalt the virulence beyond certain limits. For the rabbit 0.3 of a loopful and for the guinea-pigs 0.05 of a loopful were the smallest doses which had lethal effects in spite of many passages. The pyocyanic bacillus is therefore very different in this respect from microbes like pneumococcus and streptococcus, and resembles bacteria like typhoid bacillus.

Immunity against *Bacillus Pyocyaneus*.

A large number of experiments were carried out on rabbits for

Experiment 2.

1905.

- Jan. 9.—Rabbit received 10 c.c. autolysed filtrate.
 14.— " 10 c.c. autolysed filtrate.
 26.— " 20 c.c. autolysed filtrate.
 Feb. 6.— " $\frac{1}{10}$ c.c. toluolised broth culture.
 14.— " $\frac{1}{3}$ c.c. toluolised broth culture.
 24.— " $\frac{1}{2}$ c.c. toluolised broth culture.
 Mar. 2.— " $\frac{1}{2}$ c.c. broth culture ; killed at 60° C.
 14.— " 1 c.c. broth culture ; killed at 60° C.
 24.— " $\frac{1}{2}$ loop virulent culture ; rabbit died in 14 days with paralysis.
 24.—Control 1. $\frac{1}{2}$ loop living culture ; dead in 2 days.
 " 2. $\frac{1}{3}$ loop living culture ; dead in 5 days.
 " 3. $\frac{1}{10}$ loop ; ill ; recovered.

Experiment 3.

- April 4.—Rabbit received 5 c.c. autolysed filtrate.
 11.— " $\frac{1}{2}$ c.c. broth culture ; killed at 60° C.
 22.— " 1 c.c. broth culture ; killed at 60° C.
 May 1.— " $\frac{1}{2}$ c.c. toluolised broth culture.
 11.— " 1 c.c. toluolised broth culture.
 22.— " $\frac{1}{10}$ loop living culture.
 29.— " $\frac{1}{4}$ loop living culture.
 June 5.— " $\frac{1}{2}$ loop living culture.
 5.—Control rabbit, $\frac{1}{2}$ loop living culture—dead 2 days.
 5.— " $\frac{1}{4}$ loop living culture ; ill—recovered.
 June 14.—Rabbit received 2 loops living culture—died in 4 days.

The bactericidal power of the serum of the immunised rabbit was tested by Wright's method on June 12th, with the following result:—

Immune Serum.		Culture Dilution.	Result.
1	+	1 in 10	Growth.
1	+	1 in 100	"
1	+	1 in 1000	"
1	+	1 in 10,000	"
1	+	1 in 100,000	"
1	+	1 in 1,000,000	"

(171)

The serum, mixed in equal parts with a 1 - 1,000,000 dilution of the living culture failed to destroy the growth. The number of bacilli in the 1 - 1,000,000 dilution, as estimated by Wright's method, showed—

10 mm ³ of 1 : 1,000,000 culture dilution,	1 colony.		
10 mm ³ of 1 : 1,000,000	"	0	"
10 mm ³ of 1 : 100,000	"	13	"
10 mm ³ of 1 : 100,000	"	17	"
			} Average 15.

Thus the serum had exerted no bactericidal effect *in vitro*, inasmuch as not one single pyocyanic bacillus had been destroyed. As the serum was examined direct from the body of the rabbit, it would appear to be unlikely that *in vivo* the bacilli are destroyed by bactericidal action, as has been maintained by Wassermann.

The results of all the immunisation experiments have failed to show anything like the degree of immunity obtained by Wassermann.

As was mentioned above, Ghéorghiewsky came to the conclusion that pyocyanic immunity owed its power to the destruction of the bacilli by the phagocytes. Wright and Douglas have, however, shown that the blood fluids play a cardinal rôle in the production of phagocytosis, and on testing the sera of rabbits immunised against the bacillus pyocyaneus it was actually found that the opsonic power of the serum may be considerably greater than that of normal animals. Such immune serum as we have seen possesses no bactericidal power *in sensu strictiori*.

A few experiments were also made with pyocyanine isolated from cultures. The cultures, rich in this pigment, were shaken up with chloroform, the pyocyanine passing into solution. By allowing the chloroform to evaporate, the well-known blue crystals were obtained. As was found by Ledderhose, Charrin and Legros, pyocyanine prepared in this way presented no toxic properties. I have not been able to confirm the statement of Charrin and Ghéorghiewsky that immune serum inhibits pyocyanine formation.

Conclusions.

1. Guinea-pigs are highly sensitive to the action of bacillus pyocyaneus.

2. The filtrates obtained from living cultures of virulent pyocyanic bacilli are not toxic to guinea-pigs and rabbits except in enormous doses.

3. Bouillon cultures killed by toluol are toxic to guinea-pigs and rabbits.

4. The toxicity of such cultures is largely referable to endotoxins which have diffused out of the bodies of the bacilli. Some of the toxic action may also be due to products developed from the medium in which the bacillus is growing.

5. The degree of immunity induced by living or dead cultures is not high, the animals seldom being able to withstand more than a few lethal doses of the living culture.

6. The blood serum of animals immunised with living or dead cultures possesses no bactericidal properties when tested *in vitro* upon cultures of the bacillus.

7. The serum of animals immunised with dead or living cultures shows an increased opsonic effect in comparison with normal serum.

8. The recovery from pyocyanic infection in rabbits is probably due to phagocytosis, preceded by the opsonic action of the serum.

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ON EPIGNATHUS.

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On Epignathus.

THE condition known as epignathus is comparatively rare and of much interest.

The study of its ætiology is a subject involving important biological and pathological problems. Windle⁽²⁶⁾ in 1899 published a paper in which he reviewed in detail the literature of the subject, and discussed the various and diverse opinions regarding the nature of this class of monstrosity.

Since then, on the one hand, the work of Marchand⁽¹⁷⁾, Bonnet⁽⁷⁾, Wilms⁽²⁴⁾, and E. Schwalbe, has altered our opinions as to the teratomata, while, on the other hand, the experimental embryological work of Driesch⁽¹⁰⁾, Wilson⁽²⁵⁾, Spemann⁽²²⁾, Loeb⁽¹⁶⁾, O. Schultze⁽²¹⁾, Bataillon⁽³⁾, has broadened our view by the artificial production of malformations, more especially of double formations.

Recently, E. Schwalbe⁽²⁰⁾ has expressed the opinion that the epignathi, and those formations related to them, merit detailed work. Looking on epignathus as a connecting link between the true double formations and the teratomata, by the study of these we hope to obtain hints as to the origin and nature of epignathus.

Although records of cases of epignathus are to be found scattered throughout literature, still a large proportion of the cases are very imperfectly described. Many of the specimens have been more or less decomposed, and unfit for detailed microscopical examination; or the specimen has only been examined after having been preserved as a museum specimen for a length of time, and in such cases the history has been incomplete or altogether wanting.

I have been fortunate in receiving a very perfect specimen of epignathus, which occurred in the practice of Dr. Alexander G. Gall,* of Aberdeen.

* I wish to express my indebtedness to Dr. Gall for bringing me the specimen in perfect preservation, and also for furnishing me with notes of the maternal history.

In the present paper I describe this specimen, then give a summary of the structure and relations of the epignathi, and finally review some of the more recent theories as to the origin of epignathus.

I.—DESCRIPTION OF A CASE OF EPIGNATHUS.

The maternal history of the case is as follows:—The mother is twenty-six years of age, has been married for six years, and has born three children—two males and one female—in every way normal and healthy. The duration of the present pregnancy, as calculated from the end of the last menstrual period, was only thirty-four weeks; but, as the foetus is full time, conception must have taken place previous to the last menstruation. Towards the end of the first month of pregnancy there is a very definite history of the mother having received a severe nervous shock.

No special peculiarity was noted about the foetal membranes, liquor amnii or umbilical cord. The part of the umbilical cord still attached to the foetus is normal, and shows one vein and two arteries.

The foetus is of the male sex, and well developed, the head only showing a malformation. The hair of the scalp is thick, dark, and as much as 18 mm. in length, the eyelids are open, and the free margins of the nails of the fingers project beyond the tips of the digits. Both testicles lie in the scrotum. The foetus measures in length 488 mm. from vertex to sole of foot, 320 mm. from vertex to tip of coccyx, and, with the tumour mass, weighs 3·2 kilograms. The cranial length is 120 mm., breadth 95 mm., and circumference 327 mm. There is a centre of ossification in the lower end of the femur.

The foetus has all the appearance of having been born at full time.

From the mouth of the foetus there projects a tumour mass almost as large as the foetal head. As seen from the front, the tumour is roughly pyramidal in shape, its base lying against and obscuring the face of the foetus, while its rounded blunt apex projects downwards in front of the thorax. (Fig. 1.) The surface of the tumour is roughly divisible into an upper irregular nodulated part, covered with natural-looking skin, and a smaller part below and to the left, covered by a smooth glistening membrane, varying in colour from greyish to dark red.

(Fig 3.) Between those two parts is a furrow, and along this line of junction there is a fringe of darkish hair as much as 18 mm. in length.

The only recognisable foetal parts are a single well-formed nostril and a mouth deformed, but showing lips and gums. The nostril opens upwards, and is situated between the rudimentary mouth above and the fringe of hair below. There is thus distinct evidence of a much deformed foetal head and face, hanging by means of a stalk from the mouth of the normal foetus, the abnormal head being really inverted.

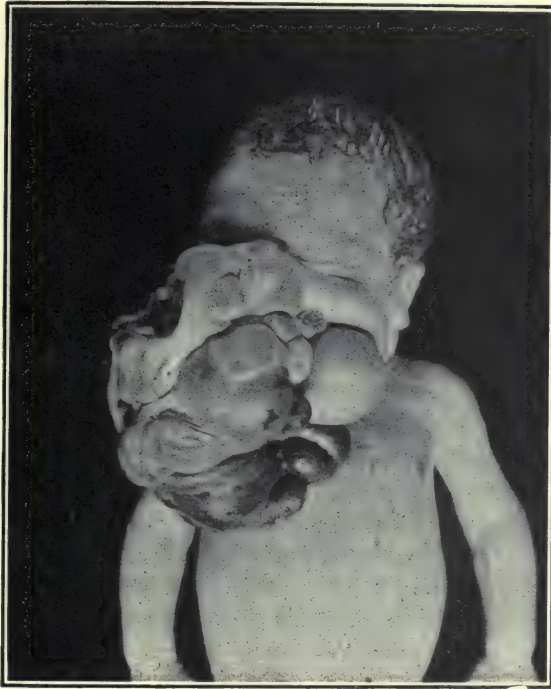


Fig. 1.—Epignathus viewed from the front and left.

This semblance becomes apparent on examining (Fig. 1) where above and to the right is seen the mouth cavity with lips and gums, and immediately under this the opening of the single nostril, and still lower down the surface covered by smooth glistening membrane, similar to that covering rudimentary brain substance in a case of anencephaly.

Viewed from the right side, the base of the tumour is closely applied to the face of the foetus, from the tip of its nose to the chin, for a distance of some 80 mm. By pushing the mass towards the left, it is seen to be attached by a stout stalk issuing from the mouth of the foetus, and this stalk of attachment is not adherent to cheeks, lips or gums.

Except for the widely opened mouth the foetal head is not deformed.



Fig. 2.—Epignathus viewed from the right side.

The greater part of the right side of the tumour is covered with natural looking skin with fine lanugo hairs. Near the centre of this surface there is an oval depressed area, 50 mm. by 38 mm. in size, the appearance of which suggests an irregular mouth cavity. This cavity is surrounded by fleshy flaps like lips, covered by skin externally and mucous membrane internally; while, again, inside these are firm con-

centric gums. These gums enclose an irregular cavity filled up with eight stalked bodies covered by mucous membrane, and from the size of a pea to that of a pigeon's egg.

The largest of these bodies has the shape and appearance of a foetal heart, while another much elongated process is quite similar to an abortive piece of umbilical cord.

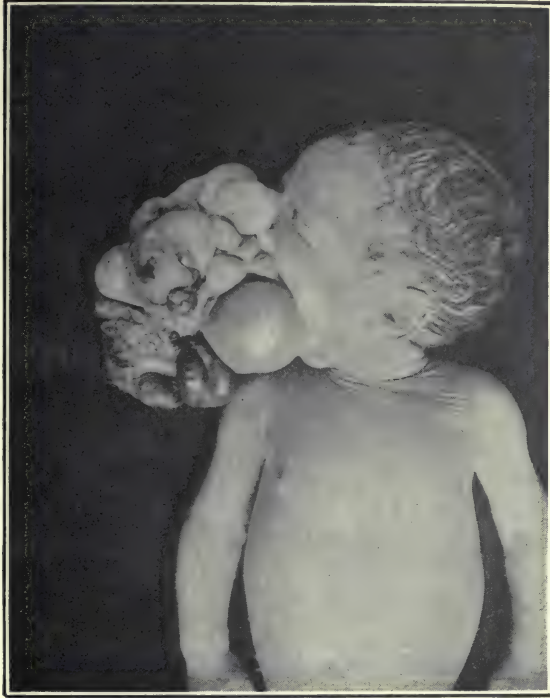


Fig. 3.—Epignathus viewed from the left side.

The left surface of the tumour mass is still more irregular and lobulated (Fig. 3). Above and behind there is one large and rounded lobule about the size of a tangerine orange, while below and in front are grouped six smaller nodules. This lobulated surface in its upper half is covered by natural looking skin with fine lanugo hairs, while the lower half is covered by a smooth membrane of a dark red colour with no hairs.

The whole specimen was now injected with a four per cent. formaldehyde solution, and placed in a solution of the same strength for two days. Thereafter the specimen was frozen and a sagittal section made, just to the left of the middle line, through both foetus and tumour.

The mesial section of the foetus, except for the head condition, presents normal appearances (Fig. 4). The body of the hyoid bone shows a centre of ossification, and is at the level of the middle of the body of the third cervical vertebra. The cricoid cartilage lies at the level of the disc between the fourth and fifth cervical vertebrae, while the upper border of the manubrium is opposite the first dorsal vertebra. The trachea bifurcates on the disc between the third and fourth dorsal vertebrae. Thus the various structures do not lie at a lower level than usual. The thyroid and thymus glands are well developed. There is no abnormality of the heart. Dark green meconium distends the large intestine, microscopically, this shows the usual constituents of meconium including delicate lanugo hairs. The bladder is distended, reaching to the umbilicus.

Coming to the tumour mass itself we can obtain a general idea of its nature by noting the appearances presented by the sagittal section depicted in Fig. 4. The section is made just to the left of the middle line and the right face is seen viewed from the left. The lower part of the section is of a dark red colour, soft and vascular, while the upper part, lighter in colour, varies in appearance and consistency. In the centre is an irregular piece of bone in which two dental sacs are seen cut across. Above and in front of this bone is fibrous tissue containing a large lobule of fat, and covered by normal skin. Passing up into the stalk of attachment of the tumour is a large serous cavity containing a closed knuckle of bowel. The lower part of the section is largely made up of rudimentary nervous tissue, in which there is a large cyst with a thin smooth wall. Just above this cyst there is a degenerate and collapsed eyeball lined by dark pigment.

Detailed Structure of Tumour.

Each half of the tumour was now carefully and completely dissected. Many pieces of the various tissues were removed for microscopical examination. These pieces were embedded in celloidin, and sections cut

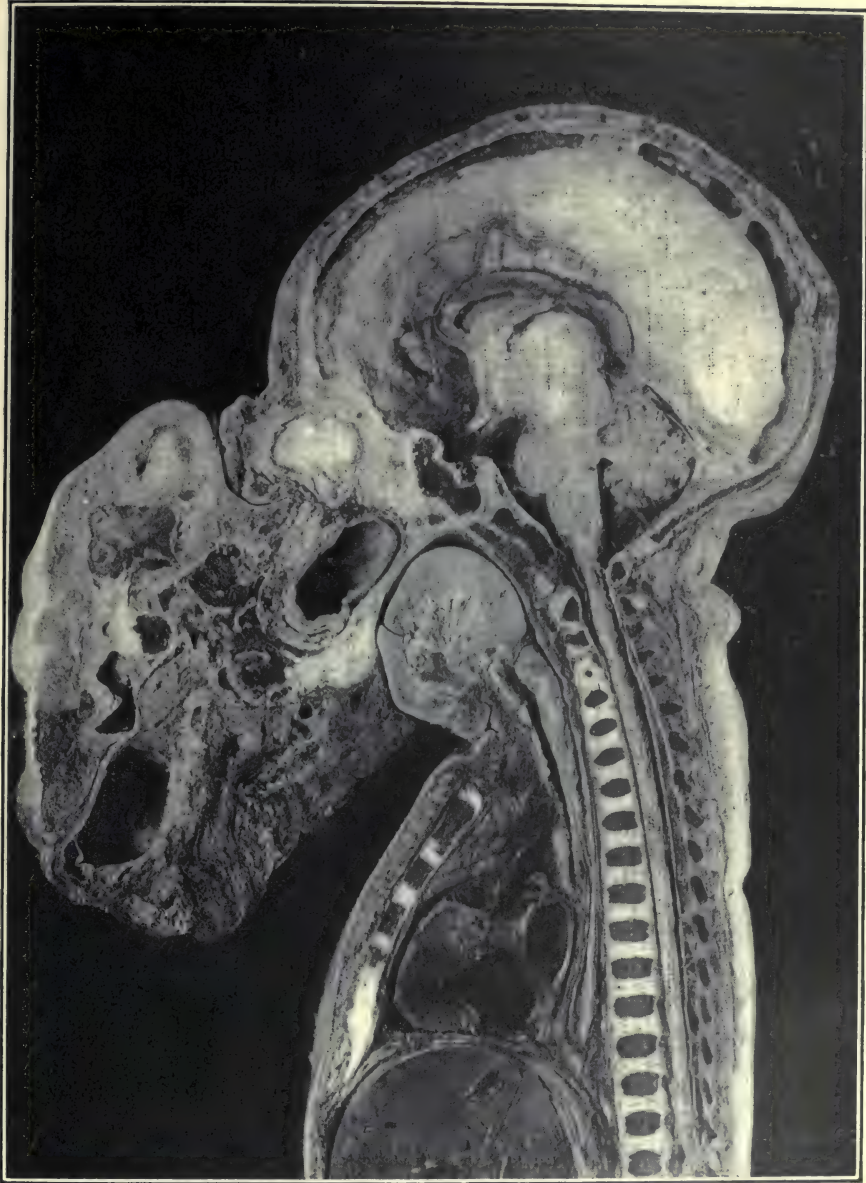


Fig. 4.—Median sagittal section of head of foetus with Epignathus.

and stained by different methods so as to establish the structure and the identity of the different tissues and organs.

The following tissues and organs are present in the tumour :—

Skin and appendages.—A large part of the surface of the tumour is covered by skin with fine hairs. Microscopic sections of this show a well-developed epidermis with numerous hair follicles and both sebaceous and sweat glands.

The lower part of the tumour is covered by a thin dark red membrane, which consists of a stratified epithelium resting on a loose fibrous tissue. Immediately under the epithelium are very numerous blood capillaries, distended with blood cells, and giving the dark red appearance to this part of the tumour.



Fig. 5.—Abortive heart attached to deformed hard palate of Epignathus. (Natural size.)

Bone and teeth.—The bone mostly forms irregular pieces radiating from the centre of the mass. To the right, one piece of bone can be identified as two fused superior maxillary bones. The palatal and alveolar processes of these bones can be distinctly recognised, and embedded in the alveolar processes are eight well-developed dental sacs containing teeth.

Mouth.—In relation with these alveolar processes is an irregular mouth cavity seen on the right aspect of the tumour (Fig. 2). This

cavity is surrounded by lips, which are covered on their outer aspect by normal skin, and on the inner by a stratified mucous membrane. No striped muscle fibre is seen in the lips.

There is an irregular hard palate covered by mucous membrane, and attached to it, and filling up the cavity of the abortive mouth, are nine stalked bodies, from the size of a bean to that of a pigeon's egg.

Abortive heart.—One of these bodies is much larger than the others, and has the appearance of a fœtal heart (Fig. 5). This measures 22 mm. in length, 12 mm. transversely, and 7 mm. from before back, and is attached by a stalk about 6 mm. in diameter. The anterior wall is quite



Fig. 6.—Abortive umbilical cord, with two large blood-vessels, attached to deformed hard palate of Epignathus. (Natural size.)

thin, and a slit made in it opens into a flattened cavity. The cavity is triangular in shape, has a slightly ridged appearance, and leads up into a vessel with a lumen about 2 mm. in diameter. An incision made through the posterior wall opens into another ventricle-like cavity. The posterior wall is from 2 to 3 mm. thick. The interior of this cavity is more ridged and irregular, and from it there also passes a thin walled channel. There is a thin septum between the two cavities, but it is not quite complete at its base.

The walls of these ventricles are made up of a loose net-work of cells with a lining of endothelial cells. No cardiac muscle fibres are seen.

Abortive umbilical cord.—Near the abortive heart, and also attached to the deformed palate of the parasite, hangs an elongated structure which has all the appearance of a piece of umbilical cord (Fig. 6). It measures 18 mm. in length, 3 mm. in diameter, and has a somewhat bulbous free end. It has the gelatinous appearance of umbilical cord, and small blood vessels are seen running along its whole length. Microscopically, it is made up of typical retiform cells, and is covered by a layer of flattened epithelium one cell deep. In the cross sections are seen four small blood vessels. The attached end of the cord passes into a mass of vascular tissue, in which break up branches from two large blood vessels (Fig. 6).

A single nostril.—Below the irregular mouth is the left half of a nose with one nostril (Fig 1). The opening of the nostril measures 3 by 2 mm., and leads into a passage 15 mm. long, bounded on the one side by a process from the deformed superior maxilla, and on the other side by an irregular piece of cartilage. The passage is lined by a thin, very vascular mucous membrane.

A degenerate eyeball.—Immediately below and deep to the nostril is a degenerate eyeball. The eyeball is firmly embedded in fibrous tissue, is somewhat crumpled, contains a clear fluid, and measures 14 mm. by 7 mm. There is neither cornea nor lens. The outer coat of the eyeball is largely cartilaginous, and inside this is a thin, vascular coat, lined by a continuous layer of typical, hexagonal, nucleated retinal pigment cells. There are no traces of the other layers of retina.

Intestine.—In the upper part of the tumour are several separate serous cavities containing irregular closed knuckles of intestine. These knuckles are distended with a clear gelatinous material, the largest knuckle having a diameter of 17 mm. A series of pieces from the walls of the different knuckles of bowel were examined microscopically. Each piece shows the four coats of normal bowel, viz., serous, muscular, submucous and mucous. The muscular coat consists of the usual two layers of fibres. Both layers are well developed, more especially the outer, which is formed of longitudinal fibres uniformly disposed. The submucous coat contains numerous small blood vessels. The mucous membrane is lined with

small cubical epithelial cells, resting directly on a well marked muscularis mucosæ. No intestinal glands are seen in any of the sections.

Salivary Gland.—Embedded in the tissue, near the deformed mouth, is an irregular glandular structure about half-an-inch in diameter. This microscopically shows the normal structure of a salivary gland. In the sections numerous alveoli and ducts are seen. Both serous and mucous alveoli are present. The mucous alveoli are not so numerous, but are readily recognisable from their clear distended mucous cells.

Thyroid Gland.—Tissue, containing thyroid gland substance, was found in an indefinite fibrous mass under the skin of the upper part of the tumour. This glandular structure has a tough fibrous capsule 1 mm. thick, and inside this a yellowish brown material, with here and there a cystic condition. Microscopically, vesicles of thyroid gland are to be made out. The vesicles are mostly elongated, and some are much distended, so that their lining cells are flattened. The smaller vesicles have walls, consisting of a single layer of cubical epithelium-cells, with deeply-stained nuclei.

Liver Cells.—In the left half of the tumour there is a solid nodule as large as a walnut. This has a smooth surface, and has the colour and consistency of liver. Microscopic sections give no indication of the lobular structure of liver, but show large cells, with the nucleated granular appearance of hepatic cells. Ramifying among these cells are numerous blood capillaries.

Nervous Tissue.—The lower part of the tumour is made up of an irregular soft mass, covered by the smooth membrane already described. On cutting into this it is seen to consist of a light yellowish material, of very friable consistency. Several pieces of this were embedded, and sections, stained by different methods, examined microscopically. All the sections agree in showing a rudimentary or embryonic nervous tissue. In the sections is seen a nuclear tissue with no cell boundaries, the nuclei lying in a syncytial protoplasm. The nuclei stain very readily, and many are dividing by mitosis. The tissue is similar to embryonic nervous tissue in an early stage of development.

Although many different parts of the tumour were examined microscopically, no nerve fibres, either medullated or non-medullated, were observed. On the right side, some fibres from the great palatine nerve were traced on to the stalk of the tumour, but only for a short distance.

Blood-Vessels.—In all parts the tumour is very vascular. In the course of dissection, several large blood-vessels were traced out, and, in the microscopic sections, numerous blood-vessels and capillaries are to be seen.

Attachment of the Tumour.

The mouth cavity of the foetus is distended by the stalk of attachment of the tumour. The lips, although compressed, are quite free, the upper lip being pushed upwards, while the lower lip is turned inwards over the alveolar margin of the lower jaw. The lower jaw is pushed downward and backward, so as to be in a plane much posterior to that of the upper alveolar margin. The mouth measures 48 mm. from angle to angle, and 40 mm. from above down.

The stalk of the tumour is attached mostly to the under aspect of the hard palate, but its posterior part is continued up along the posterior border of the nasal septum to the under aspect of the basi-sphenoid. At its attachment the stalk measures 24 mm. by 22 mm. To permit of the posterior part of the stalk passing up to the sphenoid, the soft palate is completely split into two halves. Each part of the soft palate is complete, but is compressed backwards against the lateral wall of the naso-pharynx, where each terminates in a uvula-like process. For its size and fairly extensive attachment, the stalk causes comparatively slight disturbance of surrounding parts. The cartilaginous nasal septum is complete and well developed. The left nasal fossa is normal and has well developed turbinals; the right also is little altered, but in it lies a tongue-shaped body which is attached to the stalk of the tumour, and passes forwards round the posterior border of the hard palate and extends to the anterior nares protruding as a small nodule from the nostril. This tongue-shaped body is covered with fine hairs and sebaceous material, and compresses somewhat the turbinals of the right nasal fossa.

The floor of each nasal fossa, as seen from above, is intact, and covered by mucous membrane. On examining the hard palate, from the under aspect, its anterior half is complete, but behind it is prolonged down on the sides and front of the stalk, as thin, bony laminæ. Thus when the stalk is removed the hard palate is deficient behind over an area measuring 18 mm. from before back and 21 mm. from side to side.

The stalk of attachment consists of a core of fibrous tissue on which are prolonged delicate laminæ of bone from the hard palate, and also from the vomer which is represented by two thin separate bony laminæ on each side of the nasal septum. In the fibrous tissue is embedded a piece of bowel. On its outer aspect the stalk is covered by mucous membrane. Between the mucous membrane and the laminæ of bone the main blood vessels for the tumour pass down. On the left side two blood vessels of fair size pass down from the posterior palatine foramen to the tumour, where they form a profuse vascular network. Along with these blood vessels, some bundles of the great palatine nerve can be traced on to the stalk for a short distance.

Another large artery—as large as the radial artery in the adult—issues from the left sphenopalatine foramen, crosses over the roof of the left nasal fossa, gains the posterior border of the nasal septum, and descends in the thickened tissue behind this into the right half of the tumour.

Under the mucous membrane of the posterior part of the stalk a large thin-walled vein runs up under the fibrous periosteum of the basi-sphenoid, and passing through a canal in the cartilage, between the basi-sphenoid and basi-occipital, communicates with the left cavernous sinus.

Summary of the attachment and structure of the Tumour.

The tumour is attached to the posterior half of the hard palate and partly to the basi-sphenoid. It is of interest to note that the nasal septum is complete. The relations of the attachment are such as to suggest that the tumour in its origin might have had an early connection with the mesial nasal process, and had been carried back along with the mesial nasal process through between the developing palatal processes.

The blood supply of the tumour is mainly derived from a much enlarged left sphenopalatine artery, and also from a branch of the left posterior palatine artery. The blood is returned by two veins, one large vein opening into the left cavernous sinus, and another smaller vein, a tributary of the left posterior palatine vein.

Structurally, the tumour is one of the higher forms of epignathus, containing recognisable foetal organs. A peculiarity of the specimen is,

that it shows secondary stalked structures attached to the deformed palate of the parasite. The question arises as to whether these are to be reckoned a secondary epignathus.

Representatives of all three embryonic germ layers are present, as is shown in the following table of structures identified :—

ECTODERMAL.	MESODERMAL.	ENDODERMAL.
Skin.	Connective tissue.	Intestine.
Hairs.	Fat.	Thyroid gland.
Sebaceous glands.	Cartilage.	Liver cells.
Sweat glands.	Bone.	
Embryonic nervous tissue.	Smooth muscle.	
Retinal pigment cells.	Blood vessels.	
Teeth.		
Salivary gland.		

II.—ON THE STRUCTURE AND RELATIONS OF THE EPIGNATHI.

According to Ahlfeld (¹), Hoffman, in 1688, was the first to describe a foetus with a tumour hanging from its mouth. To this condition Geoffroy Saint-Hilaire (¹³) gave the name "epignathus," defining it as a faulty developed second head which is attached to the palate of the principal head.

Ahlfeld (²) says, that, by "epignathus," one understands an *acardiacus amorphus* which stands in connection with the mouth cavity, mostly with the hard palate of its twin brother. According to his view, all swellings from the mouth of the foetus are derived from a second foetus, even although there are no evident foetal parts.

More recently, Windle (²⁶) gives the following definition. "An epignathus, then, is a congenital tumour, teratoid in its nature, or possessing recognisable parts of a foetus, which is attached within the cavity of the mouth or upper part of the pharynx." As he points out, the definition must include all forms, from the simple hairy polypus of the pharynx to the tumour with fully developed portions of a foetus.

Wittenberg (²⁷) gives a somewhat wider meaning to "epignathus," and employs it for all teratomata, for which we have to accept, that the first

(190)

place of origin has been in the stomodæum. Thus would be included amongst the epignathi certain teratomata which lie outside the dura mater in the middle fossa of the skull, the germ of which may have been in connection with the buccal evagination of the pituitary body.

E. Schwalbe ⁽²⁰⁾ arranges the epignathi in four chief groups, at the same time admitting that there are transitional forms between the several groups. These groups are as follows :—

Group I.—A second fœtus is attached, by its umbilical cord, to the palate or near the palate of a fœtus. This second fœtus may be more or less well developed.

Group II.—From the mouth of a fœtus there hang parts of the body of a second fœtus, which parts can readily be recognised as fully formed portions of a fœtus, *e.g.*, lower extremities, external sexual organs, etc.

Group III.—Out of the mouth of a fœtus there projects a formless mass in which no parts similar to fœtal organs are to be recognised. Microscopic examination gives the structure of a teratome.

Group IV.—A larger or smaller tumour is found attached to the palate or in the mouth cavity of the fœtus. Microscopic examination shows that the tumour is composed of several tissues, and is of the type of the mixed tumours.

Examples of Group I. are very rare. Ahlfeld describes and illustrates a case recorded by Baart de la Faille. In this case there was a typical epignathus attached to the base of the skull of a practically normal fœtus. In addition to this, there were two acardici acephali—pelves with lower extremities, with fork-like umbilical cords attached to the palate of the first well-formed fœtus.

Examples of Group II. are more common. The case now described belongs to this group as it shows quite recognisable fœtal parts—a nostril, mouth. Wasserthal describes an epignathus with a lower extremity attached. Otto ⁽¹⁹⁾ describes the case of a male fœtus with an epignathus showing male external genital organs. Kreutzmann ⁽¹⁵⁾ records the case of a female fœtus with an epignathus showing female external genital organs.

In examples of Group III. there are no recognisable organs, although the tumour is usually more or less covered by epidermis, and, like a teratome, may contain fibrous tissue, cartilage, bone, muscle, gut

epithelium, rudimentary brain substance, etc. To this belongs Schwalbe's case I., which had the structure of a typical teratome, and contained derivities from all three germ layers.

Group IV. includes the most simple forms, namely, the hair-clad pharyngeal polypi. These usually consist of some connective tissue and fat, with perhaps a piece of bone, and are covered by cutis and epidermis, with hairs and glands. Thus Arnold describes the case of a girl of 13 years, from whose soft palate a hairy polypus was removed. The polypus was mainly composed of fat, and was covered with skin, showing hairs and sebaceous glands, but no sweat glands.

As regards the site of implanation, we find that in the larger proportion of recorded cases of epignathus, the attachment is either to the palate or to the superior maxillary bone. This is seen on examining the list of cases published by Windle. In rather over one-fourth of the cases the tumour has a connection with the base of the skull. In a few cases the attachment has been to the soft palate or some part of the lateral wall of the pharynx.

Of those attached to the base of the skull, the majority have no connection with the interior of the cranium. However, in a small number of cases the stalk of attachment has some relation to the interior of the cranium. In Windle's case there was a tubular stalk leading through a patent pituitary foramen into the interior of the skull. In a case recorded by Hill (¹⁴), a branch of the internal carotid artery passed through a canal to the tumour. In our own case, a large vein from the tumour passed through the base of the skull to open into the cavernous sinus. In two cases recorded by Baart de la Faille, the stalk of the tumour passed distinctly through the pituitary foramen to obtain attachment to the sella turcica. In Wittenberg's case the pharyngeal swelling is continued through an opening in the sphenoid bone directly into the cavity of the skull. Another variety is where the tumour on the outside is connected, by a stalk through the sphenoid, with a tumour inside the skull, as in a case recorded by Breslau and Rindfleisch (⁸). Müller (¹⁸) describes an epignathus attached in the mouth of a fœtus, while quite separate from it lay a tumour in the right middle cranial fossa. Finally, a case is recorded by Beck (⁵), where a teratome was confined entirely to the pituitary fossa. Wittenberg argues that all such cases are to be included among the epignathi.

Thus in their attachment the epignathi point to the first settlement of their germ having taken place in the stomodæum.

III.—ON THE ORIGIN OF THE EPIGNATHI.

In looking for the cause of the disturbance of normal development which produces an epignathus, there is little suggestive in the ætiology. Out of the twenty-two cases noted, the mother was a primipara in three, and a multipara in nineteen. There is no evidence of any association with previous twins or malformed fœtuses, or of any hereditary tendency. In several of the cases, as in that now recorded, there is a history of the mother having received a severe nervous shock early in pregnancy. Hydramnios is a frequently associated condition, but this may be more an effect than a cause.

In the discussion of theories which relate to the origin of the epignathi, we find that two views have been advanced as to its development.

According to one view, epignathus is a single malformation—that is, arises from the body of the fœtus.

The generally accepted view, however, is that epignathus is to be looked on as a double malformation—the two components being asymmetrical, the one (autosite) being practically normal, and the other (parasite) incomplete and attached to the first.

Thus Ahlfeld holds the view that an epignathus is derived from a second fœtus, even although there are no evident fœtal parts—that is, he regards it as a double malformation. His theory is that there are two embryonic areas, one in front of the other, that one embryo lags in development and comes to lie under the forebrain vesicle of the other and eventually in the stomodæum, its allantois becoming fixed to the mouth and hence acardiacus. He postulates two unequal embryonic areas with their heads towards each other.

Arnold, in discussing the question of the origin of these tumours, divides them into two groups. "Autochthonous" teratomata, such as the hairy pharyngeal polypi, he traces back to the abnormal development of a "dislocation" of embryonic material in the mouth or pharyngeal cavities—that is, they are derived from one embryo. The "heteroch-

thonous" teratomata, which have recognisable parts of a second foetus, he admits have origin in a second embryo.

Marchand (¹⁷) looks on the higher foetus like forms of epignathus as asymmetrical double malformations. He draws attention to the fact that epignathus is a parasite in intra-amniotic relation with the autosite. Hence he says there must have been an unequal division of a single embryonal anlage.

He thinks the origin of epignathus is most likely from the fertilisation and development of a polar body.

Bonnet (⁷) upheld this hypothesis, but went further, suggesting the possibility of the belated division of one or more blastomeres, in an early stage of segmentation.

Wilms (²⁴) also supported the same view with regard to teratomata of the ovary and testis, and held that certain details of their structure pointed to an origin from blastomeres.

Windle, in speaking regarding the mass of germ plasm out of which the major forms of epignathus are developed, suggests the theory that "it is not the equivalent of a germ plasm of full embryo-producing power, but that it possesses varying and more limited potentialities of development."

Beard (⁴) applies his doctrine of multiple germ-cells to explain the origin of tumours. He considers that the early blastomeres of a metazoon form "an asexual foundation or larva, the phorozoon, upon which the germ-cells, and with these an embryo, take their origin." Normally, one of these germ-cells undergoes development, but two or more may develop. If two primary germ-cells "develop either together or at different times, but with abnormalities on the part of one, there may result a more or less rudimentary embryo, an embryoma of Wilms, a tumour."

E. Schwalbe accepts the view that the higher foetus forms owe their origin to a blastomere, which in an early stage was disturbed from the development of the autosite. He allows the possibility of an origin out of germ material of a later development stage. He points out that the prospective potency of blastomeres always becomes more circumscribed with progressive development. Through an unequal division of the blastomere material, or through a disturbance of a blastomere at a

time when it still has the potency to form a whole embryo or part of such, a parasitic malformation can originate.

If at first the development of such a blastomere "sists" it becomes "displaced," and may lie in various regions of the developing embryo.

With progressive development there is a decrease of the potencies of the several blastomeres. Hence Schwalbe argues that the more complicated the structure of an epignathus, the earlier must be placed the teratological process to which it owed its origin.

Accepting the view that epignathus is an asymmetrical double malformation, we have a certain amount of experimental work bearing on the cause of the disturbance of normal development giving rise to such.

Thus there is evidence to show that, by the total separation of the first two blastomeres of a single ovum, duplicate twins can result. Incomplete separation of the first two blastomeres of a single ovum may give rise to a symmetrical double malformation. All varieties of these symmetrical double malformations result according to the extent of separation and relative position of the blastomeres.

In the production of an asymmetrical double malformation like epignathus it may be that, as held by Wilder (²³), there has been a complete separation of the first two blastomeres of a single ovum, and then a fusion of the later blastomeres owing to close proximity.

Possibly, however, such asymmetrical malformations are due to an incomplete separation of a blastomere or blastomeres in a later stage of development—perhaps at a stage when such blastomere or blastomeres have not the full potentiality of a complete embryo.

O. Schultze (²¹), in working with the eggs of amphibians, found that among over-ripe eggs there was a larger proportion of double formations. Over-ripe eggs tend to break up into non-nucleated pieces, and perhaps these may become fertilised and produce a double formation. Boveri has shown that a blastomere, either with or without a nucleus, can be fertilised and develop.

Polyspermia has been accepted by some as an origin of double formations; the researches of O. Hertwig (¹²), Driesch, Fol and others negative this. Besides, it has been shown that physiological polyspermia takes place in many animals.

The most probable view is that double formations originate after the

egg has been normally fertilised. Thus in lower animals, by artificial means, such as shaking, compression, increase of temperature, altering the action of gravity or changing the osmotic pressure, the early blastomeres may be separated and each give rise to an embryo. Loeb and Bataillon, by changing the degree of concentration of the surrounding medium, brought about separation of blastomeres and double malformations. Hence it has been suggested that the production of double malformations is favoured by pathological changes in the mucous membrane of the Fallopian tube and uterus, causing alteration in its secretions.

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A CONTRIBUTION TO THE PATHOLOGY OF
EXOPHTHALMIC GOITRE.

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A Contribution to the Pathology of Exophthalmic Goitre.

THE pathology of exophthalmic goitre, despite the extensive researches which have been carried out upon the subject, still remains somewhat obscure, although the bulk of the evidence would appear to point to a primary thyroïdal origin.

The theories which have been advanced from time to time with regard to the pathology of this disease may be roughly classified under the three following headings:—(1) The thyroid theory. (2) The central nervous theory. (3) The cervical sympathetic theory.

It is very difficult to explain the various symptoms of Graves' disease upon a purely nervous origin, although many of them can be reproduced experimentally by lesions inflicted upon the great nerve centres. Filehne and Bienfait (Bull. Acad. roy. de med. de Belg., Bruxelles, 1890) were able to imitate certain of the symptoms of the disease by lesions made experimentally upon the restiform body.

Mendel (Deutsche med. Wochenschrift, Feb., 1892) has recorded a case of Graves' disease in which atrophy of the restiform body was found after death.

Nor can lesion of the sympathetic system, whether irritative or paralytic, explain all the various phenomena. Schiff, who destroyed the sympathetic nerve fibres accompanying the blood vessels of one lobe of the thyroid gland in the dog, found that both lobes remained identical in structure.

The *post mortem* appearances found in the nervous system have been extremely inconsistent and irregular, and have resembled in many cases those produced by toxic influences. Clinically, however, there would appear to be a very intimate connection with the central nervous system: the frequent development of emotional disturbances throughout the course of the malady has from the first been recognised. Sudden nervous influences, such as shock, moreover, have a very marked effect

in aggravating the symptoms, and indeed are often associated with their commencement.

On the other hand, evidence continues to accumulate in favour of the primary implication of the thyroid gland. Möbius (*Die Basedow'sche Krankheit*, Wien, 1896), Gauthier (*Lyon méd.*, 1895, lxxx., 5-12), Renaút (*Courrier méd.*, Paris, 1895, xlv.), Greenfield (*British Med. Journ.*, 1893, Dec. 9), and Kocher (*Mitteil. aus den Grenz. der Med. und Chirurg.*, 1902, Bd. ix., p. 1) all support this view.

Certain pathological alterations are invariably found in the gland, whether the enlargement be evident or slight. The number of glandular elements becomes vastly increased, and the secreting epithelium changes its character. The whole appearance of the gland suggests "a proliferation for the performance of increased function" (Greenfield). The older theory that the goitre was probably due to dilatation of the vessels has not received support from the numerous examinations made of its structure. Of course, with this increase in its tissue, and probable increase in its function, the gland becomes more vascular, still extreme vascularity is rarely an outstanding feature, and hence the enlargement of the gland can in no sense be regarded as due primarily to this cause. Edmunds ("Pathology and Diseases of the Thyroid Gland") has pointed out that when a portion of the thyroid gland is removed, the remaining part undergoes compensatory hypertrophy. The vesicles enlarge, and tend to become oval or branched; the lining membrane becomes convoluted; the secreting cells, instead of maintaining their cubical shape, become columnar. These changes are almost identical with those found in the goitre of Graves' disease, and would certainly appear to point to an increased activity of the organ. With the hyperplasia of glandular tissue there is usually some change in the microscopic characters of the colloid in the vesicles; it appears to become more mucinoid. According to Greenfield, fibrous overgrowth may ultimately ensue, which, in advanced cases, may spread from the septa into the lobules, and produce extensive atrophy.

The hyperplasia of glandular tissue and the evidence of increased activity of function suggest the probability that the disease is due to excessive formation of thyroid secretion, and much clinical evidence can be brought forward in favour of this view. The striking contrast between the symptoms of Graves' disease, and those of myxœdema, would lead

one to infer a corresponding difference in the pathology of the two affections, for while the condition of the gland in Graves' disease is most likely one of hypertrophy and increased functional activity, that prevailing in myxœdema is undoubtedly one of atrophy, with corresponding loss of function. The beneficial results following immediately upon operative procedures for the removal of portions of the hyperactive thyroid tissue also support this view, namely, that the disease is probably due to over-secretion.

Further, the condition of thyroidism, produced by over-dosing with thyroid extract, resembles Graves' disease in many of its symptoms.

As just remarked, the secretion of the exophthalmic thyroid is in all probability not normal colloid. The microscopic appearances of the material in the vesicles are certainly not those of normal secretion. Granted that there is an increased functional activity of the glandular tissue, as the character of the lining epithelium of the vesicles and the analogy with the changes seen in compensatory hypertrophy would seem to indicate, then the scantiness of the colloid contents of the alveoli most likely would point to a rapid and extensive absorption of this altered secretion, and its absorption might account for the varied nervous and other symptoms of the disease. The pathological changes discovered in the central nervous system and sympathetic might also be due to this cause; they appear to resemble the lesions known to be the result of toxic poisoning.

The following account of the condition of parts found in the thyroid gland and elsewhere, is founded upon the examination of three instances of the disease. In Case 1, permission was fortunately obtained for a complete *post mortem* examination. The portions of gland in the two other cases were excised during life; that described under Case 2 is particularly interesting, as illustrating the changes which had taken place nearly two years after operation.

Case 1.

This case was the only one which afforded opportunity for a complete *post mortem* examination. The patient was a young girl, aged 16, who had suffered for about two years from all the typical clinical features of exophthalmic goitre. She died very suddenly after over-exertion,

apparently from cardiac syncope: for a short time previously she had exhibited signs of aortic disease. At the *post mortem* examination, which was performed some twenty-four hours after death, the following appearances were noted:—

The body was somewhat emaciated. The limbs were rigid. The dependent parts were moderately livid. The pupils were equal and of medium size. The exophthalmos, which had been so evident during life, had almost vanished.



Fig. 1.—The Thymus Gland.

Heart.—The cavity of the left ventricle was dilated, and its wall was slightly hypertrophied. The aortic valve was incompetent to the water test. The edges of the two aortic cusps were puckered and calcareous. The edge of the mitral valve presented a somewhat thickened appearance, and was at one point also calcareous. The left auricle was slightly dilated.

Lungs.—The pleural surfaces were free from adhesions. With the exception of some slight hypostatic congestion at the bases, the lungs were of normal appearance.

The Spleen weighed 223 grammes. It was distinctly larger than usual. There was a well-marked notch in its anterior border. The pulp was firm and fleshy; its cut surface was of a deep red colour, dotted over with very prominent grey Malpighian bodies.

The liver, kidneys, gastro-intestinal tract, and internal genital organs were of normal appearance.



Fig. 2.—The Thyroid Gland.

The Brain, with its membranes, presented nothing abnormal to naked-eye examination.

The orbital vessels were not dilated, but the orbital cavities contained somewhat more fat than in health. The cervical sympathetic ganglia were also of normal appearance.

The Thymus Gland (Fig. 1) was enormously enlarged. It extended downwards in the form of a tongue-shaped mass, adherent to the

(205)

anterior surface of the pericardium. The upper portion of the gland consisted of two finger-like processes, the tips of which were just observable above the suprasternal notch. The left innominate vein was firmly adherent to the posterior surface of the mass. The entire gland measured $4\frac{1}{2}$ inches in length; it varied in breadth from $1\frac{1}{2}$ to $2\frac{1}{2}$ inches. The weight was 46 grammes. The cut surface was faintly granular, and had a greyish-pink colour.

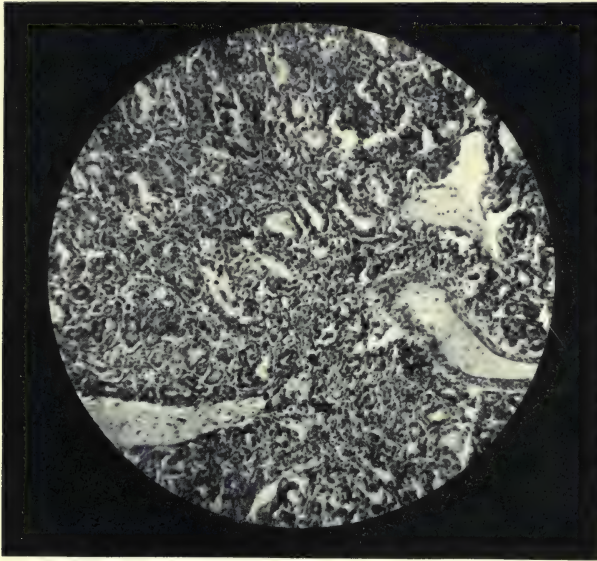


Fig. 3.—Section of Thyroid Gland (Case 1).

Shews the small size of the alveoli and the scantiness of colloid. Zeiss Obj. A, Oc. 4.

The Thyroid Gland (Fig. 2) was also greatly enlarged, the enlargement being confined to the lateral lobes, and practically symmetrical, while the isthmus had remained unaffected, and was about the size of a crowquill. The tip of the lobe on each side lay about an inch below the upper border of the thyroid cartilage; the base on each side was nearly an inch below the lower border of the cricoid cartilage. The right lobe measured $2\frac{3}{4}$ inches long by $1\frac{3}{4}$ broad. It weighed 39.5 grammes. The left lobe measured $2\frac{1}{2}$ by $1\frac{5}{8}$ inches, and weighed 29 grammes. The cut

surface of the gland was of a dark pink colour, and presented a granular fleshy appearance. There was no evidence of any increase in size of the parathyroids.

Microscopic Examination.

Thyroid Gland.—There was an undoubted increase in the number of alveoli, which were also, as a rule, much smaller than those of the normal gland. Their size, however, varied greatly, and in shape they tended to

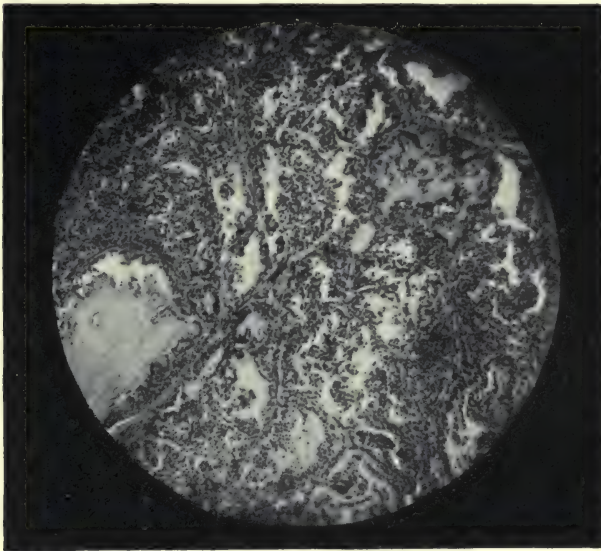


Fig. 4.—Section of Thyroid Gland (Case 1).

Shews the irregularity in shape of the larger alveoli and the papillary projections of the wall.
Zeiss Obj. A, Oc. 4.

be oval or irregular. In the case of the larger vesicles, curious papillary projections of the lining membrane were frequently seen. (Figs. 3 and 4.) The alveolar epithelium had lost its typical cubical shape, and had assumed a high columnar form (Fig. 5). The cells were stained sharply with the usual staining solutions; their nuclei were rounded or oval, and stained deeply with logwood. In one or two instances the nuclei shewed

evidence of karyokinesis, and the cells were evidently in a state of great activity. Colloid was, comparatively speaking, scanty; many of the small alveoli indeed shewed no signs of colloid in the interior. The colloid in the large alveoli had shrunk away from the epithelial lining, and shewed round its margin a vacuolated appearance. It frequently contained desquamated epithelial cells, and in some instances leucocytes. In sections stained with eosin and hæmatoxylene it usually shewed a somewhat patchy staining, which differed very markedly from the homo-

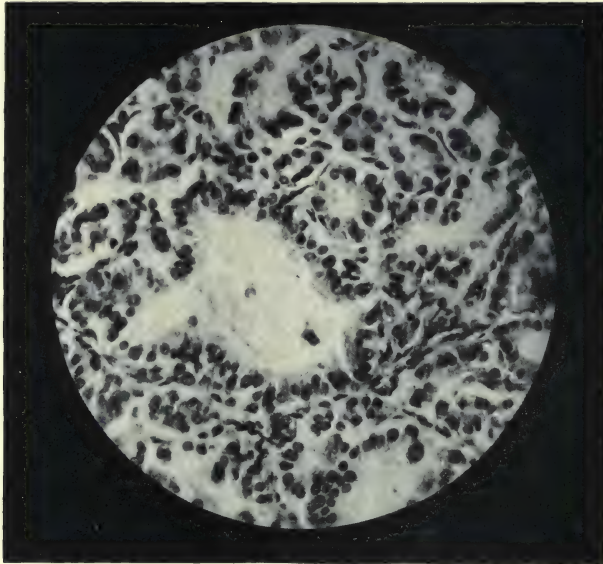


Fig. 5.—Section of Thyroid Gland (Case 1).

Shews columnar shape of the lining epithelium. Zeiss Obj. D, Oc. 4.

geneous staining of the colloid of normal thyroid. Small deposits of colloid were often seen between the alveoli, lying in what appeared to be lymphatic spaces. The blood vessels, although fairly numerous, were not dilated. The connective tissue between the alveoli was somewhat scanty. Scattered sparsely through the specimen were small deposits of lymphoid cells (Fig. 6). These deposits were of irregular shape, and consisted of a small central mass closely packed with cells, which radiated

out in short rows into the tissue between the neighbouring alveoli. These lymphoid deposits were not nearly so evident in this as in the two other specimens.

The Thymus Gland.—The condition of this gland appeared to be that of a true hypertrophy. Examined microscopically, the tissue looked like normal thymus with this exception, that the differentiation between the cortex and medulla was not quite so sharply defined. The lymphoid cells, the concentric bodies of Hassall, and the connective tissue frame-

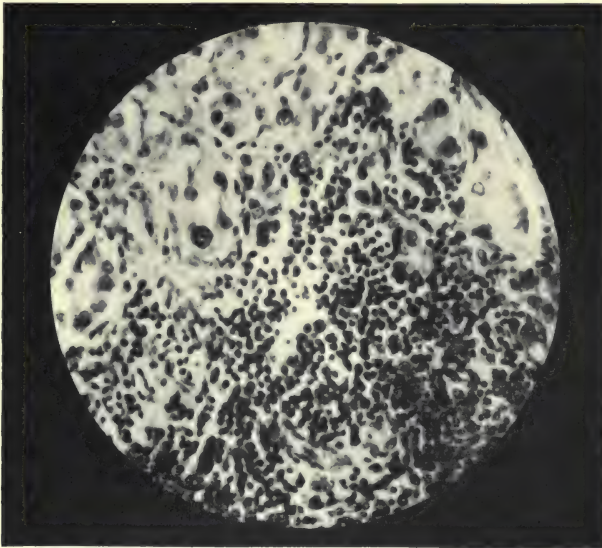


Fig. 6.—Section of Thyroid Gland (Case 1).

Shews a portion of a small lymphoid deposit. Zeiss Obj. D, Oc. A.

work were present in natural proportions. There was a complete absence of the eosinophile cells recorded by certain observers in this disease.

The Central Nervous System.—Sections were made at different levels of the upper cervical cord, the medulla, and the pons, and were stained by various methods. The parts seemed to be quite healthy.

The Cervical Sympathetic System.—Sections were made of the cervical sympathetic ganglia of both sides, but all presented a normal appearance.

The Spleen.—The Malpighian bodies were much larger than they should be, and this increase in size appeared to be due to an augmentation in the number of lymphoid cells surrounding the arterial branches, while the usual structure of the Malpighian body was in other respects retained, and the spleen pulp generally did not shew any change. Possibly this increase in the size of the Malpighian bodies may be similar in its characters to the lymphoid hypertrophy, which occurs in the thymus and other lymph-structures in this disease.

Case 2.

Clinical History.—The patient was a female, aged 19, who was first seen at the Aberdeen Royal Infirmary in February, 1904. She presented at that time a typical picture of exophthalmic goitre, the exophthalmos, rapid pulse, muscular tremor, and nervous excitability being particularly well marked.

Upon the 26th of February the right lobe of the thyroid was removed by Mr. H. M. W. Gray. In order to avoid injury of the recurrent laryngeal nerve, the posterior border of the lobe was shaved off. During the operation, bleeding was somewhat troublesome, and large vessels, both arteries and veins, could be seen running over the surface of the gland below the capsule. The patient made a good recovery from the operation, and in April, 1904, it was noted that her general condition was much improved; the exophthalmos had almost disappeared, the remaining lobe of the thyroid had much diminished in size, and the general excitability was very much less. The muscular tremors and rapid pulse still remained fairly well marked.

In January, 1906, she was readmitted to hospital. The general nervous excitability was extreme, the exophthalmos had reappeared, and muscular tremor was very distinct. The rapid pulse had again become pronounced.

Upon the 2nd of February part of the left lobe of the thyroid was removed. The gland appeared to be somewhat vascular, and bleeding was again rather troublesome.

Three weeks after this second operation it was noted that the general condition of the patient was very greatly improved. The muscular tremors had gone, the pulse was nearly normal, and the exophthalmos had again disappeared.

Examination of Specimens.

The right lobe of the thyroid, which was removed at the first operation, weighed 29 grammes. It measured $2\frac{1}{4}$ by $1\frac{1}{4}$ inches, and varied in thickness from $\frac{3}{4}$ to 1 inch. The entire lobe was enlarged symmetrically, and faintly lobulated. Its cut surface had a reddish-pink fleshy appearance; it did not seem to be very vascular, although several moderately large vessels were seen upon the capsular surface. The parathyroids had not been removed; they probably remained attached to the posterior border of the lobe.

Microscopic Examination.—The general appearances were very similar to those recorded in Case 1; there was the same tendency for the alveoli to become irregularly branched, and for the lining epithelium again to assume the columnar type. The sections, however, had a much more patchy appearance; areas of small, almost rounded, alveoli, alternating with areas of large and extremely irregular vesicles, into which they gradually merged. The lining epithelium of the spaces was high columnar in type, this character being especially distinct in the small alveoli. The small vesicles shewed in many instances an irregularly stellate mass of colloid; more frequently they were empty, or contained a small amount of granular material which stained of the same tint as colloid. The large alveoli contained much colloid matter which had shrunk away from the alveolar wall; it stained rather indefinitely, was usually vacuolated, and sometimes contained desquamated cells. The vessels were numerous and full of blood. Here and there throughout the section were small irregular deposits of lymphoid cells, in character similar to those seen in the first case. These small lymph-deposits invariably contained a few scattered plasma cells, and a small number of these plasma cells was also found in various parts of the section, generally in relation with one of the blood vessels.

The portion of the left lobe removed at the second operation consisted of two fragments. The larger of these measured 43 by 30 by 11 millimetres, and weighed $8\frac{1}{2}$ grammes. It was flat and roughly oval in shape, and its surface was slightly lobulated. The smaller fragment measured 23 by 14 by 8 millimetres, and weighed 1.9 gramme. It was about the size and shape of a small bean. The cut surface of the

fragments was of a pink or yellowish-pink colour, and presented a somewhat mottled appearance.

Upon microscopic examination the sections presented the same patchy appearance as was seen at the examination of the specimen removed at the first operation, but the areas of large alveoli now predominated. In many instances these large alveoli were of quite regular shape, and were filled with colloid, which stained homogeneously, and was of normal appearance. Their epithelium had a distinct tendency to revert to the normal cubical type, while the epithelium of the smaller alveoli still retained its high columnar arrangement. These small alveoli, however, undoubtedly contained more colloid than previously, although it still remained granular or vacuolated. Probably the most striking microscopic feature was the large amount of lymphoid tissue. Every section shewed numbers of rounded or oval lymphocyte deposits, many of them of considerable size. Occasionally one of the gland alveoli appeared to have become enveloped by the larger lymph depots, and to have undergone atrophy in consequence. Plasma cells were particularly numerous. Each of the lymph deposits contained several of them; indeed, long rows and columns of these cells could be traced between the smaller alveoli, and in parts formed quite extensive deposits. They appeared to follow the course of the capillaries and small blood vessels, and to accumulate around their walls.

Case 3.

M. W., a female school teacher, aged 41 years, had suffered from symptoms of Graves' disease for three years. Upon examination she shewed well marked thyroid tumour, distinct exophthalmos, rapid pulse, loss of flesh, and very evident muscular tremors. She complained greatly of this last symptom, and of nervous excitability and palpitation. The right lobe of the thyroid was distinctly more enlarged than the left.

On July 4th, 1903, Mr. Gray removed the right lobe of the thyroid by an operation exactly similar to that adopted in the last case. Hæmorrhage was comparatively slight, and easily controlled. She stood the operation well, and made a rapid recovery. Very great improvement in her condition resulted. Tremor almost disappeared, the exophthalmos rapidly lessened, the pulse rate fell, and the enlargement of the left lobe

of the thyroid was greatly decreased. When last seen in April, 1904, this improvement continued to be maintained.

Examination of the Specimen.

The portion removed consisted of the right lobe of the thyroid, with the exception of a thin strip along its posterior border. The lobe was enlarged symmetrically, and weighed 35 grammes, and measured $2\frac{1}{2}$ inches by $1\frac{1}{2}$. At its thickest portion it measured 1 inch. Its cut surface had the same pink fleshy appearance seen in the previous cases. Parathyroids could not be found.

Microscopic Examination.—The size of the alveoli varied greatly, and the large vesicles were, in shape, extremely irregular and branching, while papillary projections from their walls were of frequent occurrence. Colloid was more plentiful than in the former cases, but it presented the same vacuolated characters, and in many instances was granular. In every section there were a few scattered lymphoid deposits. These generally contained a small number of plasma-cells, but there were no plasma-cell-collections in the connective tissue or round the blood vessels. The connective tissue between the alveoli was more abundant than in the other cases. Blood vessels were fairly numerous, although the tissue could not be said to be highly vascular.

General Summary.

The microscopic appearance of the thyroid was essentially the same in all three cases, although it differed slightly in certain minor details. There was in each case the same glandular hyperplasia, and the same tendency to irregularity in the shape of the glandular alveoli, many of which shewed papillary projections from their walls. The columnar shape of the lining epithelium was always very marked, and the tendency towards the columnar form could be distinctly traced even in those alveoli which approximated very closely to the normal type in size, shape, and in the character of their colloid contents. The amount of colloid present, as compared with the normal, was in all cases diminished; in the smallest alveoli, in fact, colloid was usually absent. The characters of the colloid, as seen microscopically, were altered in all cases. The

small alveoli frequently contained only a small quantity of granular material, which stained somewhat deeply with eosin, and bore no resemblance to ordinary colloid; in other instances they contained a small amount of granular and vacuolated colloid. The colloid of the large alveoli was invariably vacuolated, and stained in a patchy manner; leucocytes and desquamated cells were frequently seen within it. The sections always shewed numerous lymph-deposits, in character alike with those described under Case 1. None of the thyroids exhibited any tendency to interstitial fibrotic change, and there was no evidence in support of the theory that the blood vessels played any part in the formation of the goitre.

The general appearances in all three instances were strongly suggestive of increased activity of the gland; the colloid secretion being, moreover, of abnormal character. They were in accord in most respects with the published descriptions of the changes of the gland in the disease.

Case 1 would seem to shew that, in this instance, at least, the disease was of thyroidal origin. There was a complete absence of any of the changes which have been described as occurring in the central nervous and sympathetic systems.

With regard to Case 2, the microscopic appearances of the second portion of the gland removed seemed to indicate some attempt upon the part of the thyroid tissue to revert to its normal condition as a result of the first operation. Certain of the alveoli were undoubtedly larger and of more regular shape than those seen on section of the first specimen from this case. The colloid contained in these was practically of normal character, and the lining epithelium approached very closely in some of them to the normal cubical type.

In conclusion, I must express my indebtedness to Dr. George Edmond for the opportunity he afforded me of making the *post mortem* examination of Case 1; and to Mr. H. M. W. Gray for permission to use the clinical histories of Cases 2 and 3.

THE RAT THEORY OF PLAGUE EPIDEMICS.

By WILLIAM HUNTER, M.B., C.M.,

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The Rat Theory of Plague Epidemics.

IN all the plague treatises which have been published during the past year or two, one finds a considerable amount of attention devoted to the question of the occurrence of plague infection in rats, and the dangers attached to the presence of such infected vermin in the direct vicinity of man. The dictum of Koch and many epidemiologists, that plague is primarily a disease of the rat, secondarily a disease of man, and that epidemic plague is entirely dependent on the presence of widespread plague infection in rats, has become widely recognised and accepted by many plague experts. If we look into the evidence in favour of such a conclusion, however, little information of a definite nature can be obtained. Up to the present time, no research has been accomplished which would justify the conclusion that plague-infected rats are the chief sources from which the virus is communicated to man. It must be admitted that there is much evidence in favour of such a method of transference, but, notwithstanding the constant influx of additional evidence, there yet remains to be shown the direct connection between epizootic and epidemic plague.

It is only within the past few years that the occurrence of disease in animals has become fully recognised as an important disturbing factor in the successful dispensation of modern sanitation. With the advent of more perfect knowledge in regard to the ways of infection, attention has become directed to the occurrence of diseases in animals, diseases which, when compared with those found in man, appear to be similar. The question has, therefore, arisen—a new one indeed—as to the part played by animals in the dissemination of certain diseases amongst the human race.

At the present day, one cannot take up a book dealing with plague, without being struck with the prominent part devoted to rats as disseminators of infection. In fact, on the perusal of many such manuscripts, one is drawn to the conclusion that rats, and nothing but rats, are

responsible for the existence of epidemic plague. Going into the question more thoroughly, however, it is difficult to gauge exactly the premises upon which such a conclusion has been drawn. We have to consider whether rats are the only disseminators of plague—in fact, do they disseminate plague epidemics at all?—or, granting that they are responsible for the occurrence of such—are they solely responsible, or simply partners in the wholesale serving up of the infection to the human species?

Plague in rats is by no means a recent discovery. Great mortality in rats was noted by the ancients during outbreaks of plague. Classical, mediæval, and modern literature furnishes us with many interesting details in regard to the susceptibility of various animals to plague infection. The general lay opinion in regard to the matter was, that during plague epidemics an increased mortality was observed amongst animals in the infected district, and that such a death-rate was noticeable especially amongst rats.

It would be difficult to determine who propounded the rat theory of plague epidemics. The prominence given to epizootic plague, during recent years, has arisen out of better acquaintance with the bacteriological and epidemiological aspects of the disease.

In the year 1000, Avicenna noted the presence of a high rat mortality during plague epidemics. He says:—"Et de eis, quæ significant illud, est, ut videas mures, et animalia, quæ habitant sub terra, fugere ad superficiem terræ, et pari sedar,* id est commoveri hinc inde sicut animalia ebria."

Again Nicephorus Gregoras, in 1348, and Orræus, in 1771, in their Treatises on Medicine, look suspiciously upon rats as having some relation to outbreaks of epidemic plague.

Simpson, in his recent treatise on plague, and in his Report on Plague in China and Hong-Kong, has given us an excellent resumé of the beliefs held by the Chinese in regard to the prevalence of the disease in animals such as rats. It is evident that the opinion is widely diffused amongst the Chinese that there exists some relation between the epizootic and the epidemic. In their writings, the Chinese make frequent mention

* *Pari sedar*—an Arabic expression, the interpretation of which has given rise to some difference of opinion. In AVICENNÆ ARABUM MEDICORUM PRINCIPIS, Liber quartus canonis, ex Gerardi Cremonensis versione, et Andreæ Alpigi Bellunensis castigatione (Venetiis, MDXCV.), tom. II., tract. IV., cap. 3, p. 69, *pari sedar* is translated not as above but as "exire manifeste."—ED.

that during, or immediately preceding, plague epidemics, severe outbreaks of disease occur amongst their cattle, their fowls, and their house vermin.

Reports such as these are extremely interesting to investigators of the present day, who are endeavouring as far as possible to bring rats into close relationship with the occurrence of plague amongst human beings.

Within the past decade, much evidence of a more weighty character has been brought forward in support of the rat theory of plague. The experience gained by the outbreaks of plague in the seaports of Oporto, Alexandria, Sydney, Kobe, London, etc., goes far to establish some connection between rat and human plague. In these ports, as well as in others, the disease clung fast to the harbour. The neighbouring cities and villages were not, or only slightly, affected by the disease.

Again, in the grossly overcrowded and filthy cities of the Orient, the introduction of human plague has scarcely ever been followed by the establishment of an actual endemic plague focus. In such cities or ports, in which plague broke out epidemically, these epidemics would not appear to depend on the arrival of plague-infected human beings, but upon the introduction of plague-infected rats.

In different plague epidemics, grain stores, in which rats preferably lodge, have frequently become the central points of the outbreak. For example, in Bombay, plague broke out first among the Banniahs (German Plague Commission), and in Oporto the food stores appeared to be the centres from which the epidemic spread. (Kossel and Frosch.)

Of great importance are rats on board ship. Plague-infected rats may be present on board ships, without the occurrence of human plague. Further, plague rats, or the bodies of plague rats, on board ship, are probably of greater importance than a plague-infected man. The possibility of the infection of merchandise through plague-infected ship rats is of great moment, and cases of such have been reported during the past few years. According to Kossel and Nocht, merchant ships have frequently arrived in Hamburg from plague-infected ports. No cases of plague in man have occurred on board, but, on discharging the cargo, numerous dead rats were found, many of which were plague-infected. Similar instances have been found recently in Bristol, London and Hong-Kong. In such cases, therefore, it is obvious that all precautions must be exercised to prevent contact of the healthy rats on shore with those on board ship or with the infected merchandise.

The reports of English writers, during the past few years, admit the importance of the rat in disseminating plague infection, yet the majority of these investigations show that considerable difficulty is experienced in perceiving how the infection can be conveyed from the rat to man. Snow's observations note the incidence of plague in rats, but do not suggest any connection between the epizootic and the epidemic. The Indian Plague Commission does not lay any weight on plague-infected rats as agents in the dissemination of plague epidemics. Bruce Low's papers on Bubonic Plague, dated July, 1902, lead one to believe that man and the rat are reciprocally infected. No evidence is forthcoming, however, in regard to the question from *a priori* grounds. To explain the transference of the disease from the rat to man, Hankin concluded that plague in man stood in relation to the accessibility to rats, and that probably some intermediary insect was necessary to communicate the infection from rat to man. About the same time, Simond stated that the epidemicity of plague was due to migrations of plague rats, and not to human intercourse. Further, the experience of Ashburton Thompson in Sydney, during the recent outbreaks of plague, is that plague rats were the sole source from which the infection was communicated to man. Still, as this writer has remarked, considerable systematic research is yet required in order to establish definite proof that man and the rat, in the usual circumstances of propinquity, are reciprocally infective. In Sydney, every effort was made to settle the question. Much supplementary evidence was obtained, and the conclusion drawn that infected rats play an important, if not the most important, rôle in the dissemination of the disease.

At present the general trend of opinion is against the causes which have hitherto been advanced to explain epidemic plague. It must be difficult for a certain class of professional men to get away from the time-worn causes of epidemic disease. Indeed, in all text-books on plague, one still finds the old dogmas of infection most carefully reproduced, namely, the solution of the problem by a recognition of:—

- (a) The communication of the infection from the sick direct.
- (b) Indirect means.
- (c) Place Infection, etc.

Such a table of causes would amply explain the epidemicity of plague, but the adherents of such a classification of etiology would appear to

take no cognisance of the advances made by bacteriological research. As may be gathered from the most recent works on such a subject, the ways and means afforded to the *B. pestis* to produce infection, the course of plague infection, and the exact problems underlying the spread of the pest, must undergo remodelling upon the lines indicated by the results of modern epidemiology.

At the present time the general trend of opinion is against the hitherto advanced causes of epidemic plague, namely, direct and indirect mode of communication of the disease, and place infection. My own experience of plague epidemics leads me to conclude that, apart from cases of primary pneumonic plague, the dangers of one person infecting another are over-estimated, and that place infection, apart from the presence of plague-infected rats, is of no great significance.

We know that rats are highly susceptible to plague, and they readily communicate the infection to other rats. Rats, when suffering from plague, are peculiar in their habits. They leave their holes. They are apparently deprived of any sense of fear or danger when near human beings. They progress with a drunken-like gait. Convulsive movements cause them to make erratic springs into the air during ordinary progression. They die suddenly, usually from convulsions. The natives of Bombay are so frightened by the peculiar appearance of these animals, and the finding of dead rats in their houses, that they flit at once. According to Zupitza, the natives of Kisiba, in Central Africa, have the same dread of rats either suffering from this condition or dead in their dwellings.

These remarks bring me up to the general consideration of my own results. Much evidence has been advanced in favour of the rat theory, and that, when added to the conclusions drawn from my own researches, ought to bring the epizootic and the epidemic into close relationship to each other.

Previous to my arrival in Hong-Kong, no attempt had been made to thoroughly investigate the course of epizootic plague in rats. From time to time a few rats had been examined for the presence of plague infection, but these examinations, amounting to a few hundreds only, cannot be regarded as of much value, beyond establishing the fact that such a disease as "Rat Plague" existed in the Colony.

It was evident, from the investigations of Professor W. J. Simpson, that much was to be gained by regular and systematic examinations of all rats, dead or alive, and further, his experience of plague in South Africa pointed to an intimate association between the rat epizootic and the human epidemic.

Accordingly, on my arrival in Hong-Kong, arrangements were made to have as many rats as possible collected from the various Health Districts of the Colony, and forwarded to me for bacteriological examination. Liberal assistance for the methodical carrying out of this research was provided by the Government. Four bacteriologists were obtained from Japan, and assistants were requisitioned from the College of Medicine in Hong-Kong. The following bacteriological methods were employed:—Exact details as to the place where each rat was found was furnished by the Sanitary Department. The *post mortem* examination on each rat was made under antiseptic precautions, and smears, both of the heart blood and spleen pulp, were made on microscopic glass slides. These were dried, fixed, stained by the usual tinctorial methods, and examined microscopically. In almost all cases, plague infection in rats is most pronounced, the smears usually containing millions of typically oval, bipolar, plague bacilli. Unless the bacilli were typical in appearance and present in enormous numbers, a positive diagnosis was never made. If any doubt existed as to the nature of the organisms present, Gram's method of decolorisation was employed as a counter test.

From time to time, cultural tests were also made. Owing, however, to the enormous number of rats examined daily by such a limited staff, the microscopic and tinctorial methods of examination were the only possible means of arriving at a diagnosis. Subsequent to the *post mortem* examinations, all the rats were cremated in an apparatus erected in the immediate neighbourhood.

The *post mortem* and bacteriological examination of large numbers of living and dead rats, is a research which must be carried out with considerable care. The diagnosis of the presence of plague bacilli in any tissue or organ, by means of the microscope alone, is frequently one of extreme difficulty. This is all the more so, because, in the tissues of man and animals, micro-organisms occur, which by the microscope alone cannot under any conditions be distinguished from the plague bacillus.

The research is even complicated to a much greater degree in regard to the diagnosis of the presence of plague bacilli in rats.

Rats suffer from a large number of septicæmia diseases. These diseases frequently break out amongst them in epizootic form with a heavy mortality.

Among the many micro-organisms causing these epidemic diseases may be mentioned *B. Danysz*, the *B. of Schilling*, etc. Other micro-organisms, such as the *B. of fowl cholera*, *B. of swine plague*, etc., are also pathogenic for rats. Morphologically and tinctorially all the above-mentioned micro-organisms resemble the *B. pestis*. Therefore, it is evident that even though plague is prevalent among the rats in any particular city, one has to be on guard for the possible occurrence of other epidemic diseases which might account for an increased death rate at any time among them.

Since the year 1902, this systematic bacteriological examination of rats has gone on. The following figures give approximately the number of rats collected and examined, with the total found plague-infected per annum.

Year.	No. examined.	No. found infected.
1902.	117,839	2015
1903.	101,056	3744
1904.	21,907	993

Having established bacteriologically the existence of Rat Plague in Hong-Kong, the examinations were carefully continued, and the amounts of the epizootic and the epidemic noted week by week. In this way it has been found possible to plot curves of each form of the disease. Annual charts, representing the weekly fluctuations in the epizootic and the epidemic, have been prepared. In these, the epizootic is marked by an uninterrupted line, the epidemic by a dotted line.

In dealing with the results which have been obtained through following the courses of the epizootic and the epidemic, I propose to divide my investigations into the following divisions:—

I. The History of the Course and Relations of Epizootic and Epidemic Plague in Hong-Kong—

- (a) during the year 1902.
- (b) during the year 1903.
- (c) during the year 1904.

II. The Bridging of Epidemic Plague during the intervals 1902-'03 and '04.

I.—The History of the Course and Relations of Epizootic and Epidemic Plague.

(a) *During the year 1902.*

The examination of rats was commenced in April. Previous to this date it was impossible to say more than that casual examinations showed rat plague to be existent. The extent and fluctuations of the epizootic, however, were not accurately determined until my series of investigations commenced.

HONGKONG-1902.

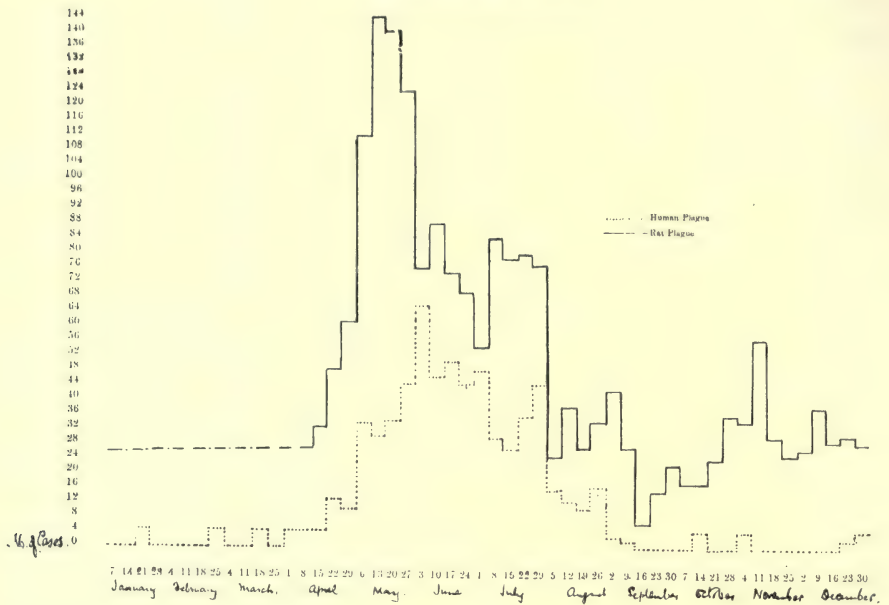


CHART I.

On examination of Chart I. for the year 1902, certain general features are at once evident. The initial rise in rat plague is a rapid one. It commences with 2, 4 or 6 cases per week. This number is doubled or tripled during the succeeding weeks, and, with gradually increasing amounts and fluctuations, the maximum of the epizootic is

reached in about a month or six weeks after its commencement. Again, it will be seen that the epizootic reaches a higher level than the epidemic.

An important point is that the epizootic appears in advance of the epidemic, the latter making its appearance a week or a fortnight later. Further fluctuations in the amount of the epizootic are usually followed by fluctuations in the epidemic, and a general resemblance of the two curves to each other is reproduced. It is interesting to note that the interval of a week or a fortnight between the fluctuations in the two curves is maintained until the maximum amount of both forms of plague is developed.

The apex of the curve of the epidemic is reached almost exactly a fortnight after the maximum amount of the epizootic.

With these general remarks, it will be well to examine the chart for 1902 more in detail.

The epizootic curve reaches its maximum within five weeks. The epidemic follows closely behind, reaching its maximum in seven weeks from the date of the commencement of the epizootic, *i.e.*, a fortnight later than rat plague. If the chart be examined closely, it will be found that elevations in the rat plague curve are followed by similar exacerbations in the amount of the epidemic. This picture is perhaps not so graphic in the chart for the year 1902, but, when examined along with that for the year 1903, the elevations and depressions of the epizootic curve are found to be followed by similar irregularities in the curve of the epidemic. Indeed, these variations of the one curve, followed by similar changes in the other, are so admirably reproduced (especially in the year 1903), that one would imagine that the epidemic curve in its rise and fall is too good to be true.

Such an epidemic curve, occurring ten days or a fortnight after that of the epizootic, and reproducing so admirably the variations in the amount of the latter, is, in my opinion, strong evidence of some extremely close relation existing between rat plague and human plague.

In order to bring the epidemic curve into close relation with that of the epizootic, the defervescence of the curves is of the highest importance. So far as the ascent and maximal elevation of the curves is concerned, the two curves follow each other closely. From this alone, however, one could not claim to have established a connection between the two outbreaks. All that can be said is that both occur about the same time,

but probably quite independently of each other. At least, the question would be asked, that, given these two curves, the one, as it were, lying within the other, what points in these lead to the conclusion that the one is the result of the other?

The history of the outbreaks is useful. It is seen that the epizootic precedes the epidemic by about a fortnight; that variations in the epizootic are followed by variations in the epidemic; that their maximal points more or less correspond; and, in general, a superficial glance at the curves makes one think of the extremely close relation which must exist between the two outbreaks. But it must be remembered, that rat plague may be present without the advent of human plague, and *vice versa*, and that occasionally the amount of rat plague bears no relation to the amount of human plague. These questions, and even others, must be answered before accepting the statement that in rats we have the key to the problem of plague prevention.

The general curve for the year 1902 shows the epidemic curve almost surrounded by that of the epizootic. From this chart alone it could not be concluded that the one is the cause of the other; if so, we should expect the rat plague curve to fall inside that of the epidemic curve. Shortly, if there is a connection between the epizootic and the epidemic, the latter should rise according to the elevations in the epizootic, and, with a fall in the latter, human plague should more or less cease.

The preliminary parts of these curves have been followed. They are seen to more or less harmonise. The point now to be considered is the relation existing between the epidemic and the epizootic towards the close of the outbreaks. If the latter is the cause of the former, it should fall previous to the decline in epidemic plague. In the general chart for the year 1902, the rat plague curve has just missed falling inside that of the epidemic. Here, however, we are dealing with an extremely mixed collection of results, taken from the City of Victoria as a whole. In Hong-Kong the city is divided up into a series of Health Districts. By comparing the relationships between the two outbreaks in individual districts, results of even a more convincing nature are obtained. By viewing the results according to this plan, a sufficient complement of epizootic and epidemic plague is obtained, and more accurate conclusions drawn. The dearth in the number of cases of the epidemic, and the excess in that of the epizootic vitiate what would otherwise have been a positive result.

In all the districts in Hong-Kong in the year 1902, with three exceptions (European quarter), the charts showed that the epizootic fades first, and is followed by the epidemic. Immediately the rat plague diminishes or disappears, human plague becomes more or less extinct. The accompanying Charts II., III., showing the condition of the outbreaks in the Health Districts, Nos. 9 and 10, clearly prove, to my mind, the close relationship which must exist between epizootic rat

HONGKONG DISTRICT No. 9.-1902.

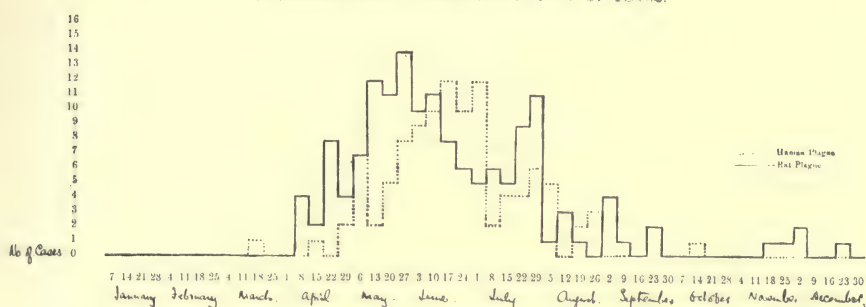


CHART II.

HONGKONG DISTRICT No. 10.-1902

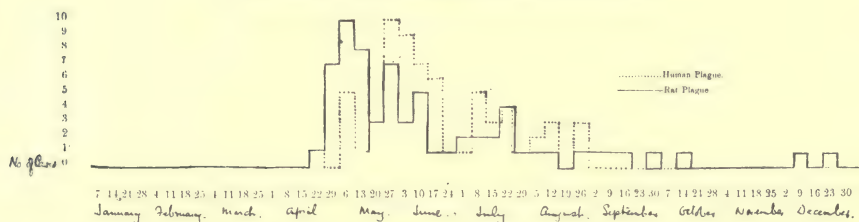


CHART III.

plague and epidemic plague. These charts demonstrate the fall in the epizootic, followed by a similar defervescence in the amount of epidemic plague.

(b) *During the year 1903.*

An important point in connection with the determination of the relation of the epidemic to the epizootic, is that, during this year, one is enabled to follow closely the train of events from the commencement of the year.

On the 1st January, 1903, rat plague was existent, and constituted more or less a definite epizootic. On the other hand, there was no trace of an epidemic. If reference be made to the curves of the epizootic and epidemic during the last quarter of 1902, it will be seen that the former raged more or less incessantly; whereas the epidemic, apart from one or two sporadic cases of plague, was non-existent. The epizootic maintained an average level of from 20 to 30 cases per week. Such a condition of affairs did not hold good for the commencement of the new year 1903. Almost immediately the epidemic appeared, and subsequently both this and the epizootic ran very characteristic charts. Within a fortnight of the new year, human plague was present (Chart IV.). The commencement of the third week of January marked the advent of the 1903 epidemic of pest. A careful survey of both curves, will enable one to draw one's own conclusions. Although the rat plague is in excess numerically, the rises and falls of the epizootic curve are followed only too closely by similar elevations and depressions in the incidence of human plague. One rarely observes such a following of one curve after another. The apices of both curves are found to correspond with the result obtained during the year 1902. Both reach their highest points within a fortnight of each other, the epizootic being first.

The course of events, after the outbreaks have reached their highest figures, is interesting. The epidemic falls in numbers so rapidly, that, practically within a month, the number of cases of human plague has fallen to an insignificant figure. But what is even more remarkable, is the sudden drop in the epizootic. The time occupied by the epizootic in falling from its maximum to its lowest level is about 15 weeks. The epidemic took about 13 weeks, namely, about a fortnight less.

The subsequent history of the curves is not so interesting. Rat plague never disappears. Human plague is practically absent. This interval between the incidence of human and rat plague appears to be more or less constant. In the year 1902, the interval between the occurrence of both outbreaks was from 10 to 14 days. The course of events during 1903 leads us to the same conclusion in regard to the time limit.

Comparing, therefore, the curves for both years we find that the epidemic begins a fortnight later than the epizootic; it reaches its maximum a fortnight later; this maximum is maintained for a similar

length of time; and its fall occupies practically 14 days. Subsequently it disappears, whereas the epizootic maintains a low but certain level. One would expect the fall in rat plague to occur somewhat previous to

HONG KONG-1903

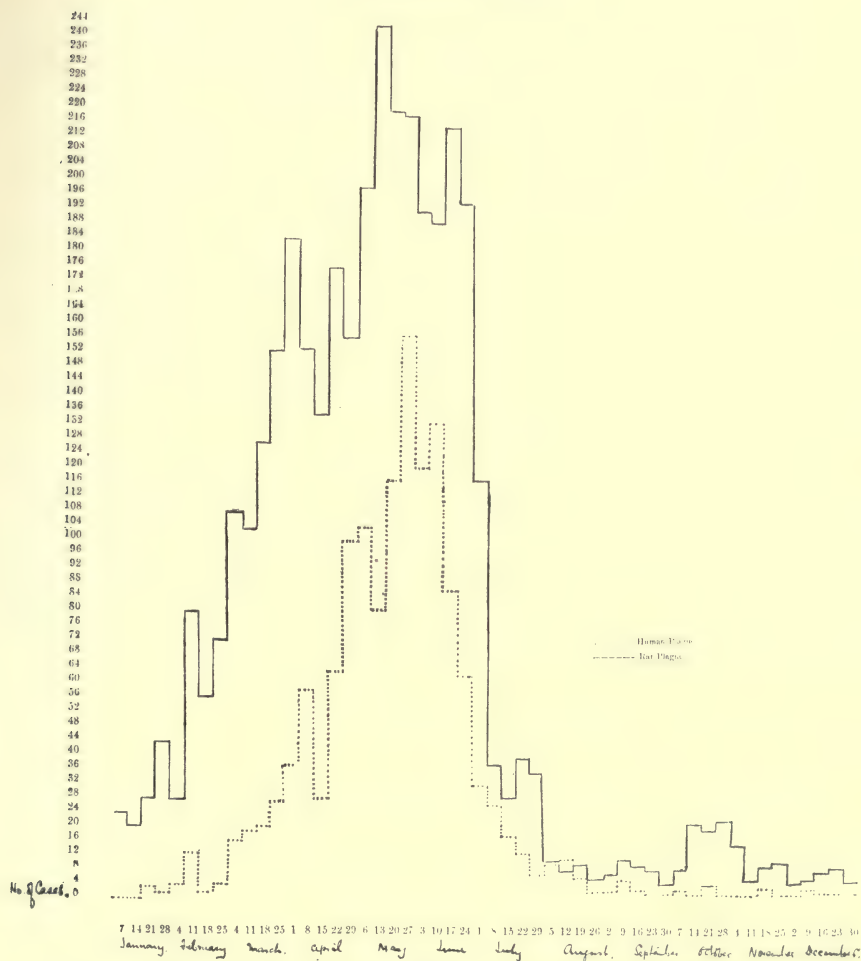


CHART IV.

that of epidemic. The condition of affairs is, however, a repetition of what took place during 1902.

Summing up these two curves, the conclusions which one must necessarily draw, are that human plague commences immediately after the increased incidence of rat plague, and that variations in the latter occasion variations in the former.

A close study of these charts is almost bound to lead one to think of some relation between the two outbreaks. It may be said that both run independently of each other, only the epidemic appears about a fortnight later. However, it would be difficult to imagine such a regular system in the incidence of an infectious disease, when compared with an epizootic. We may imagine the epidemic of plague commencing practically at the same season during each year, but that it should regularly appear within a definite incubation period after the advent of the epizootic is difficult to get away from, unless we admit of the existence of a definite relationship. Again, it is found that the plague epidemic does not commence at a definite period during each year. In 1902 it was not present until the middle of April. In 1903, it began about the 1st of February; in 1904, as we shall see later, its commencement was even later than during 1902.

If we compare these dates with that of the advent of the epizootic, the following is found:—In 1902 the epizootic began about the 1st of April; in 1903 it began about the middle of January, and in 1904 it will be found that a similar condition of affairs was present. Thus it is evident that, apart from the season of the year, epidemic plague appears to be preceded by epizootic plague, and that the interval of time between the outbreaks varies from ten days to a fortnight.

(c) During the year 1904.

The condition of affairs during this year compares very favourably with the points already alluded to under the two previous years. The attached Chart V. itself explains the constant relation between the degree of severity of the epizootic and the epidemic. During the first week in January it is seen that rat plague was present to a considerable extent in the Colony. The epizootic rose step by step, until the beginning of February, when a rather erratic jump upwards took place. This sudden increase in the amount of rat plague was followed by the appearance of human plague after about ten days.

1904.

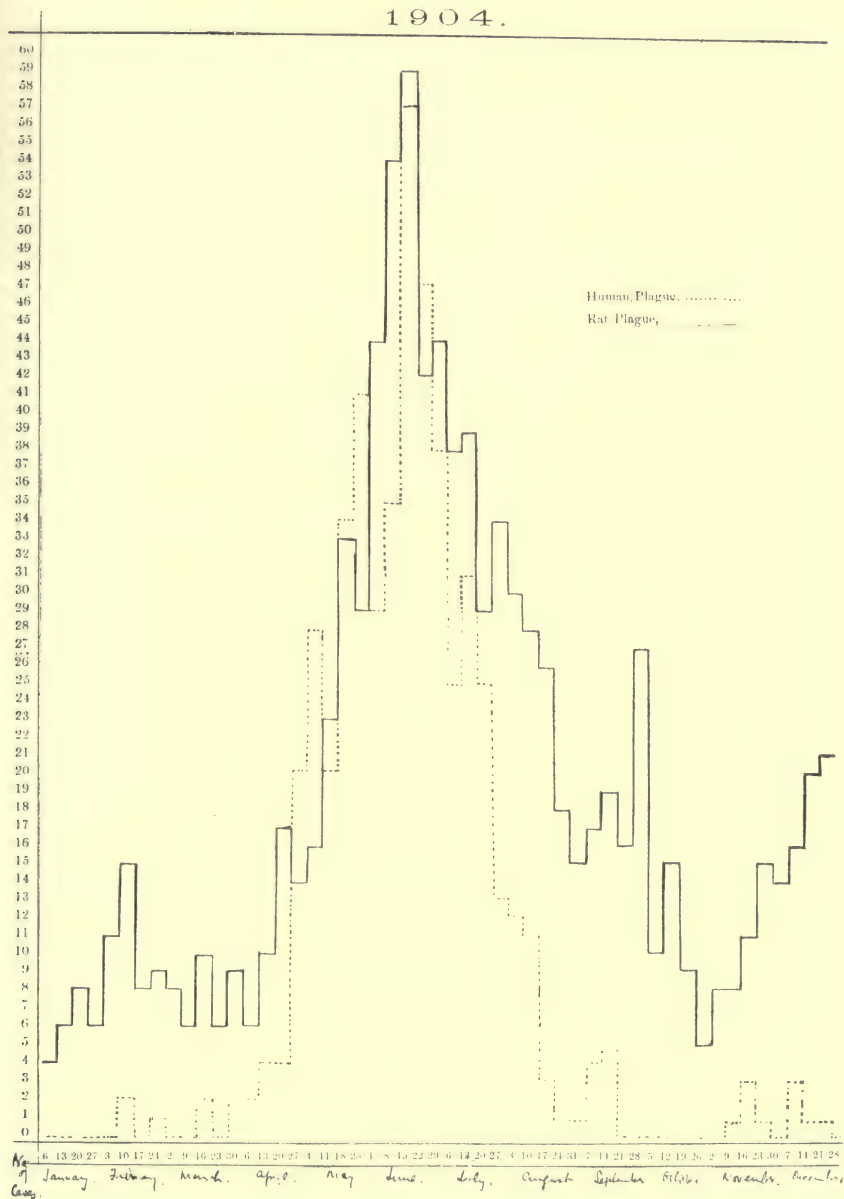


CHART V.

(231)

Further remarks in regard to the curve appear unnecessary, as a comparison of this chart with the foregoing will clear up any doubtful points.

II.—The Bridging of Epidemic Plague during the intervals 1902-'03 and '04.

In almost all plague-infected countries, the epidemic is found to be most prevalent during certain seasons of the year. In Hong-Kong, these epidemics rage from March to July inclusive. In other countries plague appears during the colder seasons of the year and *vice versa*. The reasons for such a seasonal recurrence of plague are by no means obvious, and a consultation of current plague literature helps one but little. At the present time, climatic influences would appear to have lost much of their significance. Plague epidemics occur in Siberia as well as in the equatorial regions, and carefully prepared records of sunshine, humidity, and rain shows these factors to have little determining influence on the occurrence of plague epidemics. All that can be said in regard to climate is that it may exercise an indirect influence on the course of the infection.

During the interval between the end of one epidemic and the first cases of the succeeding epidemic, human plague is, to all intents and purposes, non-existent. A few cases do occur, but these are of no great practical significance, apart from the fact that they help us to remember that with the decline of the last epidemic our sanitarians have, in all probability, not yet succeeded in stamping out the disease.

Further, the reasons for the outbreak of erratic cases of human plague are by no means clear.

To begin with, little or nothing is known in regard to the history of epizootic plague in rats through a number of years. Many authorities believe that rat plague dies out on the decline of human plague.

Two charts have been prepared, showing the relations existing between epizootic and epidemic plague during the period, July, 1902, to June, 1903, inclusive (Chart VI.); and during the period, July, 1903, to May, 1904, inclusive (Chart VII.). These show clearly the condition of affairs which obtains in an endemic plague centre like Hong-Kong. In both charts the epidemic vanishes, but the epizootic persists with a low

and oscillating curve. This curve is more regular in its course than during the epidemic periods.

From these curves, therefore, we find that rat plague is present throughout the whole year in mild epizootic form. As a result of this, sporadic cases of human plague appear from time to time. Epidemic plague, on the other hand, is prevalent only during the acute exacerbations of epizootic plague.

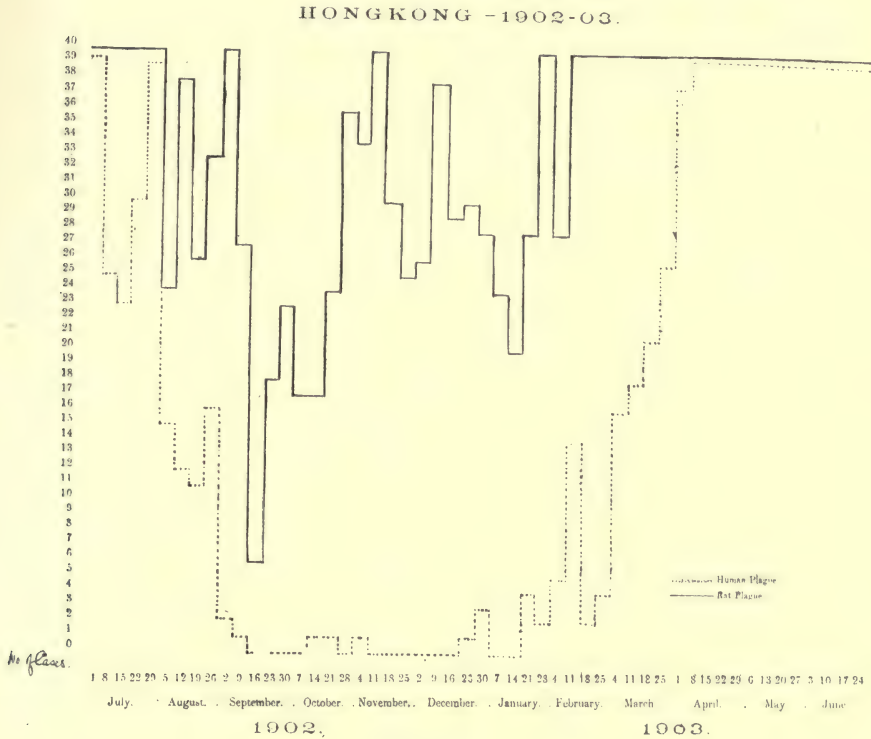


CHART VI.

Another point of importance in regard to these two charts is the following:—A comparison of the epizootic during 1902-'03 with that during 1903-'04 shows us that the former was much more severe. Secondly, the amount of rat plague at the end of 1902 was in excess;

and, thirdly, there is the fact that the epidemic of 1902 was a mild one ; that of 1903 was severe, and during 1904 the epidemic was again slight.

Given these data, coupled with a careful scrutiny of the two charts, it would appear that a malignant epizootic occurs towards the end of an epidemic, and its continuance after the disappearance of the latter means an early recurrence of human plague and a severe epidemic. On the other hand, a mild epizootic during the interval between two epidemics would appear to indicate a late recurrence of human plague and a mild epidemic. The condition of affairs may be stated as follows :—

1902.—Epidemic mild.	} The epizootic is continuous.
1902-1903.—Epizootic severe.	
1903.—Epidemic severe.	
1903-1904.—Epizootic mild.	
1904.—Epidemic mild.	

It has now been clearly shown that in an endemic plague centre like Hong-Kong, rat plague is present, in varying amount, throughout the whole year. It becomes epizootic at certain times of the year, and the reasons for this would not appear, at first sight, to be obvious.

In order to explain this regular appearance of the epizootic, I believe that a consideration of the question of the variability in virulence of the *B. pestis*, and the period when rats are most prolific, will simplify matters greatly. The *B. pestis* is an organism which is liable to sudden alterations in its virulence. Two races of the bacillus may be cultivated under the same conditions for months. One may retain its virulence. The other may lose its virulence, either partially or completely. This loss of virulence may take place suddenly. It is stated that the plague bacillus may remain virulent for a considerable length of time at the temperature of 95°-100° F.

Further, the continued passage of a strain of the *B. pestis* through rats, leads eventually to the production of practically avirulent stocks of the organism.

Such cultures are found by experiment to produce in rats and other animals a condition of chronic and marasmic plague.

Further, chronic rat plague is predominant during the intervals between epidemics of plague, and from such cases an avirulent strain of the *B. pestis* may be obtained.

My results show that :—

- (1). Acute rat plague is followed by epidemic plague.
- (2). Chronic rat plague would appear to bridge over epidemic plague.

An explanation of the occurrence of chronic rat plague, as a sequel to an epizootic of acute rat plague, is by no means easy. From what

HONGKONG - 1903-04



CHART VII.

has already been said, the continued passage of the *B. pestis* through the body of the rat tends to render the organism less virulent. Therefore, it is probable that a strain of the bacillus which excites acute rat plague during the early passages, will only cause chronic rat plague during the

later passages. In the plague bacillus we are dealing with an organism of the group of hæmorrhagic septicæmiæ. Such bacteria, when introduced into the bodies of certain animals, are known to lose their virulence progressively. They give rise to outbreaks of spontaneous disease in animals, and, on recrudescence of such epizootics, the virulence of the organism becomes increased. During the natural evolution of the epizootic, we find that the microbe becomes attenuated, and either finally disappears, or, as in plague, persists in chronic form. The recrudescence of rat plague in acute form would appear to depend upon the natural infection of fresh generations of rats, the bodies of which are highly susceptible to plague infection. My observations and knowledge of the habits of rats lead me to the conclusion that the obstinate clinging of plague to any particular area, and its periodic recrudescence, is occasioned through chronic rat plague, and further, the recrudescence of the epizootic occurs at certain seasons of the year, namely, during the period of maximal rat prolificacy.

SOME EXPERIMENTS WITH DISINFECTANTS.

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Some Experiments with Disinfectants.

THE following investigation was undertaken at the suggestion of Professor Hay, in order to obtain, at first hand, reliable information as to the efficiency of certain disinfectants, especially formaldehyde or formaline, for the guidance of the Public Health Department of the city.

A trustworthy means of gaseous disinfection has been long sought for by medical officers of health, and it is now the accepted opinion that formaldehyde is the best. The disinfectant properties of formaldehyde have been investigated by numerous experimenters, but their results have been by no means uniform. While all, or nearly all, agree as to its undoubted value as a surface disinfectant, there is a wide variance of opinion as to its penetrating power. It is admitted by all that it is virtually non-poisonous, and does not injure textile fabrics. Kenyon states that he subjected over 225 samples of different textile fabrics, hair, fur, leather, etc., to crucial tests, using solutions of various strengths and a saturated atmosphere of the gas. The results obtained were in every way satisfactory.

Formaldehyde exists in at least three well-recognised isomeric states :—

1. Formaldehyde (formic aldehyde), a gas at ordinary temperature, colourless and possessing slight odour, but having an extremely irritating effect upon the mucous membranes of the nose and conjunctiva. At a temperature of about 20° C. the gas polymerizes into paraformaldehyde, known commercially as paraform.
2. Paraform, a white substance, unctuous to the touch, soluble in both water and alcohol. It consists chemically of two molecules of formaldehyde. It is this substance which is supposed to

compose the commercial solutions of formaldehyde, known as formaline, formol, etc.

3. Trioxymethylene, formed by the union of three molecules of formaldehyde. It is a white powder, giving off a strong odour of the gas. It is but slightly soluble in alcohol and water.

Formaldehyde gas possesses about the same specific gravity as air, which renders it only slightly diffusible, although more so than sulphur dioxide, in consequence of which it penetrates more readily to all portions and corners of a room. Formaldehyde combines with nitrogenous organic matter to form new compounds. A few drops of formaldehyde added to white of egg will prevent its coagulating by heat, and it is from this faculty of combining directly with albuminoids forming the protoplasm of the micro-organisms that the gas is supposed to derive its powers as a germicide. Formaldehyde also readily unites with the nitrogenous products of decay, fermentation, and decomposition, forming new chemical compounds, which are both odourless and sterile. The commercial solutions known as formaline, formol, etc., are said to contain 40 per cent. of formaldehyde gas. They are not always up to this standard, and being volatile, there is a certain loss if they are not properly kept. In winter there is a decided deterioration owing to the polymerization and precipitation of the insoluble trioxymethylene, the gas probably existing in solution in one of its polymeric states. Formaline is probably dissolved paraformaldehyde. This is one of the reasons why the simple heating or evaporating of the solution does not always result in driving off the gas, but sometimes results merely in dissipating the water, leaving a residue of solid paraform. Trillat has shown, however, that this polymerization is prevented if the solution is heated under pressure.

A.—GASEOUS DISINFECTION WITH FORMALDEHYDE.

Temperature, as with disinfectants in general, is an important factor in disinfecting with formaldehyde. Disinfection with this gas should not be attempted if the temperature is under 10° C., as the gas tends to condense to paraform at lower temperatures. In cold weather it is

recommended that the room to be disinfected should be heated by artificial means, high degrees of heat aiding the disinfectant powers of the gas. A certain amount of moisture has also been found to be essential to successful gaseous disinfection, and the maximum disinfecting power is got only if the atmosphere be saturated with moisture. H. W. Hill states that an amount of gas which failed to kill in 6 hours at 42 per cent. humidity, killed in 20 to 40 minutes at 100 per cent. Another important requirement is that the gas be evolved rapidly, so as to obtain a sufficient concentration of the gas in the room at one time. Rideal, in his experiments with Thursfield's lamp, found that a larger amount of the disinfectant rapidly evolved is of greater value than prolonged exposure to a smaller quantity of the gas.

Many methods have been given as the most reliable for fumigation with formaldehyde gas. For this investigation three methods were chosen, viz. :—

1. Lingner's apparatus, as representing the method of gaseous disinfection with the atmosphere saturated with moisture.
2. The "Alformant" Lamp, where the method is dependent on the natural humidity of the atmosphere at the time of the experiment.
3. The Permanganate Process, as recommended by the Sanitary Authorities of the State of Maine, U.S.A.

1.—Lingner's Disinfecting Apparatus.

This apparatus, which is made in Germany, consists of a ring boiler in which steam is generated and driven into a reservoir filled with formaline or glyco-formol (30 per cent. formaldehyde with 10 per cent. glycerine). By the pressure of the steam the formaline is ejected in the form of a fine and very abundant spray through four separate nozzles out of the reservoir. Two litres of glyco-formol are recommended as sufficient for a room of 2800 cubic feet capacity. The addition of glycerine is supposed (1) to hinder polymerization, (2) to hinder evaporation of the formaline after deposition in the room, (3) to thicken the solution and favour its adhesion to a smooth surface and its absorption by pores, in consequence of which its effect is believed to be enhanced. The

addition of glycerine has since been found to be unnecessary, the employment of the 40 per cent. formaldehyde solution being quite as efficacious.

Koch, in 1898, from a series of comparative experiments with different methods of gaseous disinfection, concluded that the Lingner method was superior to the other methods of gaseous disinfection. He found that, after $1\frac{1}{2}$ hours' exposure, *Bacillus anthracis* and *Staphylococcus pyogenes* were killed; that faeces exposed on a shelf was sterilised, but that faeces in the pocket of a coat gave positive results. These satisfactory results, coming, as they did, when plague was prevalent, led many sanitary authorities on the continent, especially in Russia, to adopt it as a means of disinfecting plague-infected houses.

In this country, Lingner's apparatus is not yet well known, and relatively few scientific tests have been made of its efficiency. Accordingly, attracted by the continental reports of its value, Professor Hay, four years ago, procured the apparatus for use in the work of the Public Health Department of the city, and the immediate incitement to the present inquiry was his desire to have it tested under conditions that would reproduce, as nearly as possible, those under which the work of disinfection of a room and its contents has to be carried out in Aberdeen. The following series of experiments with the apparatus were designed to ascertain not only (1) its power as a disinfecter, and (2) its powers of penetration, but also (3) to test how far these good results were obtainable where no special precautions were taken to seal up every aperture in a room, further than roughly stopping up the chimney, as often happens in ordinary fumigation.

EXPERIMENT I.

The first experiment was carried out in an old working-class dwelling, forming part of a three-storey tenement in the heart of the city, and consisting of one principal room, with two small rooms or sleeping closets opening off it. The large room measured 18 ft. by 11 ft. by 8 ft., and had an open fireplace and two windows, both windows being loose in their frames. Two panes of glass had been broken, and were roughly covered with paper tacked to the frames. The entrance door of the room fitted very loosely, there being a considerable space

between the foot of the door and the floor. The two smaller rooms measured 9 ft. by 7 ft. by 8 ft., and 8 ft. by 7 ft. by 8 ft. respectively, and each had one small window but no fireplace. The total cubic space of the main room and the two closets amounted to 2536 cubic feet.

The object aimed at was the disinfection of the whole dwelling, the apparatus being placed in the middle of the floor of the main room, while the doors leading to the small rooms were left open. During the experiment the four windows and the door were closed in the usual way, without sealing, but the chimney was roughly stopped with paper.

Technique.—Small blocks of unvarnished wood, each 4 in. long by 1½ in. broad, and having a small oblong area marked on the surface, were sterilized in an autoclave at a temperature of 120° C. for one hour.

The following micro-organisms were used in the experiment :—

1. an actively sporing culture of *Bacillus anthracis*.
2. 24 hours' culture on agar of *Staphylococcus pyogenes aureus*.
3. 24 hours' culture on agar of *Bacillus coli communis*.
4. 24 hours' culture on agar of *Bacillus typhosus*.
5. 24 hours' culture on serum of *Bacillus diphtheriæ*.

Emulsions of these different micro-organisms were made with bouillon in sterile test glasses, and spread, by means of sterile brushes, on the marked areas of the wooden blocks, and allowed to dry in air. They were then distributed in the following places :—

1. floor of the main room.
2. the mantelshelf above fireplace of main room.
3. ledge above window of main room.
4. shelf, 7 feet from floor, directly opposite the apparatus.
5. floor of one of the closets, in a corner remote from the door.

In this experiment the blocks were protected from direct deposition of the disinfectant by means of several loosely placed layers of brown paper, and were exposed to the action of the vapours from the apparatus for 3 hours. The marked areas on the wooden blocks were then scraped by means of sterile chips of glass, and the scrapings were placed in tubes of bouillon, and allowed to incubate for 7 days at a temperature of 37° C. Control blocks made at the time of doing the experiment were treated in a similar way, except that they were not exposed to the vapours.

Within 24 hours a copious growth had taken place in the tubes prepared from the control blocks. On the other hand, all the tubes containing the material which had been exposed to the vapour from Lingner's apparatus remained sterile even after 7 days.

EXPERIMENT 2.

The second experiment was carried out in a small ward of the City Hospital, Aberdeen. It had 3 close-fitting two-sashed windows and a stove, the outlet of which was closed by stuffing with paper. The only other precaution taken was to suspend a wet sheet outside the door to assist in preventing any fumes reaching the patients in the adjoining wards. The size of the room was 16 ft. by 17 ft. by 10 ft., giving a cubic capacity of 2,720 cubic feet, or just 80 feet under the maximum allowed by Lingner for the adequate action of the apparatus. The apparatus was placed in the middle of the floor.

Technique.—Small pieces of glass, 1 in. long by $\frac{1}{2}$ in. broad, were sterilized in the autoclave. The following micro-organisms were used :—

1. actively sporing culture of *Bacillus anthracis*.
2. 24 hours' agar culture of *Staphylococcus pyogenes aureus*.
3. 24 hours' agar culture of *Bacillus coli communis*.
4. 24 hours' agar culture of *Bacillus typhosus*.
5. sputum containing the tubercle bacillus in large numbers.

The cultures were made into emulsion with bouillon, and spread on the sterile glass slips. The sputum was spread on an uncovered Petri's capsule. All were allowed to dry in air. They were then exposed in the following manner for three hours to the action of the vapours from the Lingner apparatus :—

1. One set of the four cultivations and one specimen of the sputum were placed on the floor, and protected from direct deposition of the vapour by several loose layers of paper.
2. One set of the cultivations alone were set on the mantelshelf above the stove.
3. One set of cultivations and one of sputum were placed between two blankets—that is, having the thickness of two blankets above and two below.

4. One set of cultivations and one of sputum were placed in the centre of a thick mattress, which had been opened up for the purpose and re-closed.

Control experiments were at the same time carried out.

After 3 hours' exposure, the slips of glass were transferred direct into bouillon tubes, without any intermediary process, and incubated at 37° C. Within 24 hours, growth occurred in all the control tubes, as also in all the tubes from the 4th set; while the tubes from the 1st, 2nd and 3rd sets remained sterile after 10 days' incubation. The sputum, from exposures Nos. 1 and 3, were each mixed up with a small quantity of bouillon, and inoculated into guinea-pigs—the sputum from No. 4 being rejected owing to the negative results from the cultivations similarly exposed. After an interval of ten weeks, both guinea-pigs were killed, and in neither case was there the faintest trace of a tubercular lesion.

Conclusions.—From these experiments, conducted as they were under conditions which severely tested the powers of the apparatus, it will be seen that Lingner's disinfecting apparatus gives trustworthy results in the disinfection of dwelling-rooms, and, as shown by the result of sets 3 and 4, the penetrating power of the disengaged vapour, although not sufficient for mattresses or other thick goods, is apparently quite effective for lighter articles of bedding and clothing.

In the working of the apparatus, I found it advantageous, in order to obtain an immediate and full effect, to begin with very hot water in the boiler. It is also essential to see that all the four jets are working freely.

2.—The "Alformant" Lamp.

The Alformant Lamp had also been employed for some years in the Public Health Department of the city, its use being confined to certain cases where its convenience was an inducement. It was, therefore, suggested by Professor Hay that I should also test its efficiency, the more so as its makers ascribed to it a high disinfecting power, and the lamp was widely advertised and used.

This method of evolving formaldehyde gas consists in the heating of paraform, one of the polymeric forms of formaldehyde, which first melts and then breaks up into the two molecules of formaldehyde gas. The

lamp is cheap and light, and of very simple construction, consisting of a receptacle or basin for holding the paraform, or, as they are commonly called, the "formaline tablets," with a small methylated spirit lamp beneath. The disadvantages of the method are that the gas is given off without moisture, and tends to polymerize readily, especially on cool, dry days. The gas is also given off rather slowly, and with little force, so that it permeates slowly to all the crevices and corners of a room. The room to be disinfected must have all cracks and crevices carefully sealed up.

Dr. Kenwood, in a paper read at Leeds, in 1897, stated that he had succeeded, by means of the lamp, in sterilizing swabs infected with *B. diphtheriæ*, using 21 tabloids in a room of 2,004 cubic feet, with a four hours' exposure. More recent experiments, under varied conditions, seem to have given very mixed results, and Kenwood now advocates using 25 tablets per 1,000 cubic feet, if disinfection is to be ensured. Allan, in 1898, in a series of comparative experiments with sulphur dioxide and formaldehyde, conducted in two ordinary dwelling-rooms, used 10 tablets for each 1,000 cubic feet, as recommended by the makers, and got satisfactory results. He found (1) that it was not so much a question of time as of initial volume of gas liberated, and suggested using a large number of lamps rather than a large number of tablets in one lamp; (2) that the gas had no great power of penetration; and (3) that the generating apparatus should be as near to the floor as possible.

The following experiment was conducted with the view of comparing the Alformant lamp method with the Lingner method. In order that the comparison might be as fair as possible, the house used in experiment 1 was employed, and the same conditions were adhered to, viz., merely stopping up the chimney. In order that the initial volume of the gas should be as large as possible, I used two lamps, with 30 tablets in each, giving approximately 24 tablets per 1,000 cubic feet. The relative humidity of the air before starting was 86 per cent.; the inside temperature was 56° F. The organisms used in this experiment were (1) actively sporing *Bacillus anthracis*, (2) 24 hours' culture on agar of *Staphylococcus pyogenes aureus*, (3) *Bacillus typhosus*, and (4) *Bacillus coli communis*.

Technique.—Small pieces of glass, 1 in. long by $\frac{1}{2}$ in. broad, were sterilized in the hot air chamber. Emulsions of the different cultures

were made in sterile test glasses, and thereafter spread on the slips of glass with a sterile öse, and allowed to dry in air. Control slips were made, and also allowed to dry in air, and then put inside a sterile Petri dish, and kept for the same time as those exposed to the disinfectant. The other slips were distributed in the house as in experiment 1, viz., (1) on floor behind the main door, (2) upon the shelf, 7 feet from the floor, near to the lamp, (3) upon the mantelshelf above fireplace, (4) upon the ledge above the window, and (5) on the floor of one of the smaller rooms, in a corner remote from the door. They were then exposed to the action of the Alformant lamps for $3\frac{1}{2}$ hours. The room, on being opened at the end of the time, had a powerful odour of formaldehyde gas. The different slips were then placed direct into bouillon tubes and incubated for 7 days at 37° C., the control slips being at the same time placed in bouillon tubes. Within 24 hours, a copious growth was present in all the control tubes. At the end of 7 days, the following results were observed in the tubes containing the exposed slips:—

In the 1st set (floor of main room) growths were observed in the tubes inoculated with Anthrax, Staphylococcus, and B. coli, while the B. typhosus tube remained sterile.

In the 2nd set (shelf near to lamps) negative results were obtained in all the tubes.

In the 3rd set (mantelshelf above fireplace) growths were obtained in the Anthrax and Staphylococcus tubes, while both the B. coli and typhosus tubes remained sterile.

In the 4th set (above window) growths were obtained in all the four tubes.

In the 5th set (floor of smaller room) growths were obtained in all the four tubes.

Conclusion.—This experiment shows that, where the gas is present in sufficient quantity, as in the 2nd set of organisms, disinfection will be obtained; but it cannot generally be relied upon in ordinary conditions, even where the proportion of paraform used, as in the foregoing experiment, is considerably above that recommended by the makers of the lamp. It also shows that B. typhosus is the most susceptible of the exposed germs to the action of the gas, three out of the five samples of the cultures of this bacillus being sterilized.

Of course, these indifferent results were obtained in the case of a room which was by no means hermetically sealed, but it was in the same condition as when the corresponding test was made with Lingner's apparatus; and it is well not to rely on any very careful sealing of rooms in the large number of fumigations which have to be carried through by sanitary officers in times of heavy epidemics. The better results obtained by some other observers were no doubt largely due to the more careful sealing of the rooms.

3.—The Permanganate Process.

This method is based on the fact that, by the mixture of formaline and potassium permanganate, a copious liberation of gaseous formaldehyde takes place.

The State Board of Maine recommends the following method:—In the centre of the room is placed a large vessel containing the requisite amount of potassium permanganate. The proper quantity of formaline is then added. As much foaming occurs, it is well to place the vessel within a large pan.

For each 1000 cubic feet, 13 ounces of potassium permanganate and 2 pints of formaline are used. It may be mentioned that the New Hampshire Board, who have also employed this method, considers that smaller quantities of the substances ($3\frac{1}{4}$ ounces of potassium permanganate and 1 pint of formaline to the 1000 cubic feet) serve the purpose equally well.

Experiments were carried out by the Maine authorities on the following micro-organisms:—*B. diphtheriæ*, *B. typhosus*, *B. coli*, *B. pyocyaneus*, *B. anthracis*, *B. subtilis*, *Staphylococcus albus*, *S. aureus*, *Streptococcus pyogenes*, &c. After an exposure of 3 hours to the action of the disinfectant, out of 1529 test objects, only 27 showed any growth, and of these, 21 were *Bacillus subtilis*.

In these experiments the ordinary openings of the room were closed, but in no case were precautions taken to close up cracks and crevices.

The following is a description of my own experiments with these methods:—

EXPERIMENT I.

This experiment was carried out in the same house as that used in experiments with the two last-mentioned methods, and the same

conditions were adhered to. It was conducted in all respects according to the recommendations of the New Hampshire Board, $2\frac{1}{2}$ pints of formaline and $8\frac{1}{2}$ ounces potassium permanganate being employed. The temperature of the air was 51.5° F., and the relative humidity was 83 %.

The organisms used in this experiment were :—

1. actively sporing *B. anthracis*.
2. 24 hours' culture on agar of *Staphylococcus pyogenes aureus*.
3. *B. typhosus*.
4. *B. coli communis*.

Technique.—The technique employed was similar to that of the previous experiments, that is, the exposure of glass slips coated with dried emulsions of the various organisms. Control experiments were made at the same time with similar slips kept in a sterile Petri dish in the laboratory.

The other slips were distributed in the house, as in experiment 1, viz. :—

1. on floor, behind main door.
2. upon the shelf, 7 feet from the floor, near the vessel.
3. upon mantelshelf, above fireplace.
4. upon ledge, above window.
5. on floor of one of the smaller rooms, in a corner remote from the door.

They were exposed to the action of the disinfectant for 3 hours. The exposed slips, and the control slips, were then placed direct into bouillon tubes, and incubated at 37° C. Within 24 hours growth was present in all the control tubes. At the end of 7 days, the following results were observed in the tubes containing the exposed slips :—

In the first set (floor of main room) growths were observed in the Anthrax, *Staphylococcus*, and *B. typhosus* tubes, while the *B. coli* tube remained sterile.

In the 2nd set (shelf near to vessel) growth was observed in the Anthrax tube, while the other tubes were sterile.

In the 3rd set (mantelshelf above fireplace) growths were observed in the Anthrax and *B. typhosus* tubes, while the *Staphylococcus* and *B. coli* tubes remained negative.

In the 4th set (above window) growths were observed in the Anthrax and *B. typhosus* tubes, while the *Staphylococcus* and *B. coli* remained sterile.

In the 5th set (floor of closet) growths were obtained in all the four tubes.

EXPERIMENT 2.

The second experiment was carried out in a small ward of the City Hospital, Aberdeen. It had 3 close-fitting two-sashed windows and an open fireplace, the chimney of which was loosely packed with sheets of newspapers. The temperature of the room was 54° F. The capacity of the room was 2,890 cubic feet. It may be noted that in this experiment the proportion of formaline was doubled, 5 pints of formaline and $16\frac{1}{4}$ ounces of potassium permanganate being used.

Technique.—This was in all respects similar to that of the last experiment, the micro-organisms used being :—

(1) Sporing Anthrax ; (2) *Staphylococcus pyogenes aureus*, (3) *B. coli*, and (4) *B. typhosus*. Three sets of these organisms were made for exposure to the action of the disinfectant, and were distributed as follows, viz. :—

1st set, on floor of room behind door.

2nd set, on mantelshelf above fireplace.

3rd set, on sash of one of the windows.

They were exposed to the action of the disinfectant for 3 hours. The room, on being opened at the end of the time, had a fairly powerful odour of formaldehyde gas, although not quite so marked as in experiment 1. The different slips were then placed direct into bouillon tubes, and incubated for 7 days at 37° C. Control slips, made at the same time as those used in the experiment, were likewise placed in bouillon tubes and incubated. Within 24 hours, a copious growth was present in all the control tubes. At the end of 7 days, the following results were observed in the tubes containing the exposed slips :—

In the 1st set (floor of room) growths were observed in the Anthrax and *Staphylococcus* tubes, while the *B. coli* and *B. typhosus* tubes remained sterile.

In the 2nd set (mantelshelf over fireplace), growth was present in the Anthrax tube, while the *Staphylococcus*, *B. coli*, and *B. typhosus* tubes remained sterile.

In the 3rd set (sash of window) growth was present in the Anthrax and Staphylococcus tubes, while the *B. coli* and *B. typhosus* tubes remained sterile.

Conclusions.—These experiments show that this method of disinfection is quite as efficient as the Alformant lamp, but the results are not so conclusive as those got by Lingner's apparatus. It has distinct advantages in that there is no danger from accidents due to fire, and that it requires no special form of apparatus.

On the basis of my experiments, the Lingner apparatus is, for ordinary disinfecting work, unquestionably superior to the Alformant lamp and the Permanganate process.

It ought to be added that, although the Alformant lamp is cheap, costing only a few shillings, the paraform tablets are much more expensive than a corresponding amount of formaldehyde in solution.

B.—LIQUID DISINFECTION BY FORMALINE AND OTHER DISINFECTANTS.

Formaline is a 40 per cent. solution of formaldehyde in water, and, as met with in commerce, is a liquid of a pale, sea-green tint, with a specific gravity of about 1.070, and an acid reaction which is due to formic and acetic acids. In more concentrated solutions, the formaldehyde tends to pass into the polymeric and comparatively inactive form. This, therefore, is the strongest solution which will remain permanent. When exposed to the air the formaldehyde vapour is gradually dissipated and the solution loses strength. Loew and Fischer, in 1886, were the first to state that formaline possessed powerful antiseptic properties. Trillat showed that the presence of a minute quantity of this substance in urine preserved it from putrefying. Buchner, Aronson, Blum, Cohn, and others, have investigated the disinfectant properties of formaldehyde in solution. All are agreed in attributing to it powerful antiseptic and deodorant properties. Blum pointed out that to kill micro-organisms it requires a somewhat strong solution. Slater and Rideal found that a 1 per cent. solution of formaldehyde is sufficient to kill *Staphylococcus pyogenes aureus* in from 50 to 60 minutes, *B. typhosus* in from 40

to 50 minutes, *B. coli* in from 30 to 40 minutes, *B. anthracis* and *Vibrio cholerae* in less than 15 minutes; while solutions, varying in strength from 1 in 5,000 to 1 in 20,000, were sufficient to inhibit the growth of most organisms. Trétrop considered a 2 per cent. solution of formaline to be a most efficient antiseptic, and stated that he obtained positive results with it after other preparations had failed. Bird speaks highly of formaline as a disinfectant and antiseptic, but finds that the vapour is more powerful as a disinfectant than the solution. Schepilewsky confirmed the experiments of Bird. Kenwood suggests the use of a 2·5 per cent. solution for spraying. Leslie Mackenzie and Alexander speak of the efficacy of a ·5 per cent. solution for general disinfecting purposes. Park and Guerard find that a 3 per cent. solution will kill Anthrax spores in 15 minutes, while a 1 per cent. solution will kill all other germs in one hour, and most germs within 30 minutes.

Hill and Abram, in their experiments on the disinfection of excreta, found that faeces when mixed with a 1 in 20 solution of formaline, remained sterile on subsequent inoculation. Houston found that formaline in a dilution of 1 in 100 did not prevent the growth of *B. coli* after one hour's contact, while in a dilution of 1 in 20 it killed that organism. Thresh and Sowden have made a series of experiments to ascertain the strength of solution necessary to ensure sterilisation. Cultures of *B. diphtheriae*, *B. typhosus*, *B. pyocyaneus*, *V. cholerae*, *M. prodigiosus*, and *Staph. pyogenes aureus* were spread on wood, on white-washed surfaces, and on wall paper. The infected surfaces were sprayed with solutions of formaline varying in strength from ·5 to 2 per cent. After three to four hours' exposure, the sterility of the sprayed surfaces was tested by means of sub-cultivations, with the following results:—The ·5 solution killed all the organisms on wood and wall paper, but failed to destroy the *B. pyocyaneus* on white-washed surfaces. The 1 per cent. solution gave similar reactions, but the 2 per cent. solution sterilised all surfaces.

Muir and Ritchie found that to disinfect an organic mixture containing pyogenic organisms, a 10 per cent. solution, acting for half-an-hour, is necessary, and that in the case of pure cultures, a 5 per cent. solution would kill the *V. cholerae* in 3 minutes, *B. anthracis* in 15 minutes, and anthrax spores in 5 hours. When such

organisms infect clothing, an exposure to a 40 per cent. solution of formaline for 2 hours, and in case of anthrax spores, an exposure for 24 hours, is necessary. Silk threads impregnated with *B. pestis* were found to be sterile after 2 minutes' exposure. Nils Englund recommended spraying rooms with a 2 per cent. solution, afterwards closing the room for 24 hours. Walter found that a solution of 1 to 10,000 arrested the growth of *B. anthracis*, *V. cholerae*, *B. typhosus*, *Staphylococcus pyogenes*, and *B. diphtheriae*, and that slightly stronger solutions sufficed to destroy these organisms. Faeces were rendered sterile by a 10 per cent. solution in 10 minutes.

The lack of uniformity in the various methods of testing the germicidal power of antiseptics and disinfectants has long suggested the necessity for the introduction of some standard method. It must, of course, be conceded that the subject does not lend itself to the exact treatment of chemical analysis. With a testing material consisting of living germs always liable to variations of vitality and degrees of resistance, *absolute* results are impossible, but, nevertheless, a degree of accuracy may be attained sufficient to render the results of great comparative value. It is evident at any rate that isolated observations upon single antiseptics and disinfectants are of little value. It is only by comparison of the germicidal action of the various disinfectants under similar conditions that one can arrive at correct conclusions. With this object in view, Ainslie Walker has proposed a means whereby a properly systematized method of estimating the bactericidal value of disinfectants may be established. He suggests that some well-known disinfectant be selected as a standard—one known to give regular and consistent results—such as pure phenol (carbolic acid). Briefly summarised, the technique of his method is as follows:—To 5 c.c. of a 24 hours' blood-heat culture in broth of the organism add 5 c.c. of the dilute disinfectant. Shake, and take sub-cultures at definite intervals in suitable media. Incubate for at least two days at 37° C. If an agar culture be preferred, take up part of the growth on the point of a platinum needle, and distribute it evenly in sterilized water; the resulting emulsion may be used in place of the broth culture, the rest of the procedure in both cases being identical. A similar experiment is carried out simultaneously with a dilute solution of phenol. From the results thus obtained, the strength or efficiency of the disinfectant is expressed in multiples of the

volume of carbolic acid performing the same work ; or this multiple may be expressed as the "Carbolic Acid Co-efficient." For example :—

B. COLI COMMUNIS—24 HOURS' BROTH CULTURE.

Disinfectant.	Dilution.	Thin Cultures exposed to Disinfectant (in minutes).						Period of Incubation.	Temperature.
		2½	5	7½	10	12½	15		
X.	1-1,000	+	+	—	—	—	—	48 hours.	37 C.
Phenol.	1-100	+	+	—	—	—	—	48 hours.	37 C.

+ = Growth ; — = No Growth.

The multiple here is 10—*i.e.*, it will require 10 volumes of carbolic acid to perform the work done by one volume of the disinfectant (X) under observation.

The following experiments were undertaken to test and compare the relative actions of Formaline, Phenol, Corrosive Sublimate, Cyllin, Izal, Sanitas, and Sapo-Cresol.

Phenol.—Carbolic acid, once the most widely used of disinfectants, has been disparaged in recent years because laboratory tests have demonstrated that it cannot be depended upon to kill spores. A 1 or 2 per cent. solution has no certain effect upon anthrax spores. A 3 per cent. solution requires 7 days, a 4 per cent. solution, 3 days, and a 5 per cent. solution 2 days, to kill anthrax spores. Weaker solutions are effective for non-sporing bacteria. Behring found that ½ per cent. solution killed the germs of Cholera, Plague, Typhoid, Diphtheria, and Erysipelas in one hour, while a 1 to 1½ per cent. solution would destroy these organisms in one minute.

Corrosive Sublimate.—The high disinfectant power of perchloride of mercury is well known. It is largely used in surgery, but it is little used, at any rate in this country, for ordinary disinfection. One important objection to its use being its great toxicity. A solution of 1 in 1000 is ample for the destruction of non-sporing bacteria in half-an-hour. Solutions of 1-500 and 1-800 will kill non-sporing bacteria in a short time. For spores, 1-500 solution, with an exposure for one hour, is necessary. Andrewes has pointed out that the resistance of the *Staphylococcus pyogenes aureus* against mercurial salts is altogether exceptional, but that this resistance does not extend to antiseptics of

other groups, such as carbolic acid, &c. He found that suspensions of the *Staphylococcus pyogenes* in sterile water were destroyed by a 1-500 solution of corrosive sublimate in $12\frac{1}{2}$ minutes, and by a 1-1,000 solution in 50 minutes, while beef-tea cultures of the organism required for their destruction with similar dilutions of the disinfectant, 45 minutes and 4 hours respectively. These results illustrate remarkably well a point upon which Andrewes has laid great stress—the fact, namely, that the presence of organic matter exercises a distinctly retarding influence on the germicidal action of corrosive sublimate.

Cyllin is the new name for creolin, as manufactured by Jeyes' Disinfectant Co. Its composition, however, is not quite identical with the fluid sold until recently as "creolin," but has been modified with a view to obtain the highest germicidal value with the lowest possible toxicity to higher animals. It is stated to contain no carbolic acid or its homologues, and to rely solely for its high efficiency on certain members of another chemical series which have been recently isolated. It forms a slightly pinkish emulsion when mixed with water.

Klein, in a series of experiments, completed after my own investigations had been largely carried out, compared the action of cyllin, formaline, and carbolic acid on *B. pestis*, and found that a solution of 1-30 formaline did not kill *B. pestis*. On the other hand, a solution of 1-80 carbolic acid killed *B. pestis* after 10 minutes' exposure, and a solution of 1-2400 cyllin killed *B. pestis* after 10 minutes'. Klein concludes from these experiments, that the germicidal power of a 1-2,400 solution of cyllin is equal to that of a 1-80 solution of phenol, and greater than a 1-30 solution of formaline. Therefore, the disinfecting power of cyllin is about 30 times as great as that of phenol, and more than 80 times that of formaline. These results are certainly very striking. It ought, however, to be mentioned that the experiments were apparently undertaken at the instance of the makers of cyllin. How far my own experiments agree with these will be seen later. They were undertaken without any communication with the makers, and the cyllin was purchased in the ordinary way.

Izal.—A non-poisonous disinfectant obtained as an oil from the by-products in the making of coke. Izal, as it appears on the market, is a 40% emulsion of this oil. It forms a light fawn-coloured emulsion when mixed with water.

Klein, in a series of experiments, found that solutions of 1-200 killed all non-sporing bacilli in 5 minutes, and a solution of 1-300 killed the *Streptococcus pyogenes*. He found that solutions of 1 per 1,000 inhibited the growth of all spores and bacilli, except in the cases of *B. prodigiosus* and *B. typhosus*, where a solution of 2·5 per 1,000 was required. Fowler obtained the "phenol coefficient" of 5·5 with *B. typhosus* and *B. prodigiosus* in the case of izal, and 8·5 in the case of cyllin. Kenwood and Hewlett obtained the "phenol coefficient" of 5·7 with *B. coli* in the case of izal, and 5·3 in the case of cyllin.

Sapo-Cresol.—A poisonous disinfectant, with a very pronounced creosote-like odour. It forms a dirty white emulsion when mixed with water. So far as I am aware, no bacteriological report has appeared on this substance. (c.f. Wolf. Arch. f. Hyg., 1894, xx., p. 219.)

Sanitas.—A pleasant odoriferous disinfectant. It contains hydrogen peroxide, camphor, and thymol. When mixed with water, it forms a clear fluid.

Thresh, in a recent series of experiments, found that a 20 per cent. solution of sanitas fluid kills *B. typhosus* in 5 minutes, and a 40 per cent. solution kills *B. diphtheriæ* in 20 minutes. Pure sanitas fluid kills *B. diphtheriæ* in 1 minute, and *B. anthracis* in $2\frac{1}{2}$ minutes. A 5 per cent. "sanitas emulsion" kills the *B. diphtheriæ* in 30 minutes. A $7\frac{1}{2}$ per cent. "emulsion" kills *B. typhosus* in $2\frac{1}{2}$ minutes. A 10 per cent. "emulsion" kills *B. anthracis* and *B. diphtheriæ* in $2\frac{1}{2}$ minutes.

The following was the procedure in the first series of the experiments made by myself. (Izal, sanitas, and sapo-cresol were not included in this series):—

1. The germs taken were cultures of *Staphylococcus pyogenes aureus*, *B. typhosus*, and sporing anthrax, and they were grown in bouillon, each tube containing exactly 10 c.c. bouillon. The first two cultures were of 24 hours' growth, and the latter of 7 days'.
2. To each of these tubes enough of each disinfectant was added to give the different strengths of solution of disinfectant determined upon. The tubes were then thoroughly shaken.
3. At every $2\frac{1}{2}$ minutes, up to 15 minutes, 7 loopfuls of the mixture were re-inoculated into fresh tubes of bouillon and incubated at 37° C for 8 days.

The cultures used had been recently isolated, the *Staphylococcus* being isolated from an acute abscess, the *B. typhosus* from a recently fatal case of typhoid fever, and the anthrax from a recent case, and in an actively sporing condition. The formaline was tested just before commencing the experiment, and was found to contain 32 per cent. of formaldehyde gas, so that the different dilutions given must be calculated upon that basis and not the ordinary one of 40 per cent. The various dilutions of the disinfectants employed were such as are in common use for disinfectant purposes.

The following results were obtained :—

FORMALINE (Strength 32%).

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).						Period of Incubation.	Temperature.
Anthrax spores.		2½	5	7½	10	12½	15	8 days.	37° C.
	1-30.	+	+	+	+	+	+		
	1-50.	+	+	+	+	+	+		
<i>B. typhosus</i> .	1-100.	+	+	+	+	+	+	8 days.	37° C.
	1-30.	+	+	+	+	—	—		
	1-50.	+	+	+	+	+	+		
<i>Staph. pyog. aureus</i> .	1-100.	+	+	+	+	+	+	8 days.	37° C.
	1-30.	+	+	+	+	+	+		
	1-50.	+	+	+	+	+	+		
	1-100.	+	+	+	+	+	+		

(+ = Growth ; — = No Growth).

Growth was present in all the tubes with the exception of *B. typhosus* in solution of 1-30 at 12½ and 15 minutes' exposure.

PHENOL.

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).					
Anthrax spores.		2½	5	7½	10	12½	15
	1-20.	+	+	+	+	+	+
	1-40.	+	+	+	+	+	+
<i>B. typhosus</i> .	1-80.	+	+	+	+	+	+
	1-20.	+	+	+	—	—	—
	1-40.	+	+	+	+	—	—
<i>Staph. pyog. aureus</i> .	1-80.	+	+	+	+	+	—
	1-20.	+	+	+	+	+	+
	1-40.	+	+	+	+	+	+
	1-80.	+	+	+	+	+	+

(+ = Growth ; — = No Growth).

Growth was present in all the tubes of anthrax spores and *Staphylococcus pyogenes*.

B. typhosus killed in 10 minutes by 1 in 20 solution, in 12½ minutes by 1 in 40 solution, and in 15 minutes by 1 in 80 solution of phenol.

CORROSIVE SUBLIMATE.

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).					
		2½	5	7½	10	12½	15
Anthrax spores.	1-1,000.	+	+	+	+	+	+
	1-2,000.	+	+	+	+	+	+
	1-5,000.	+	+	+	+	+	+
B. typhosus.	1-1,000.	+	—	—	—	—	—
	1-2,000.	+	+	+	+	—	—
	1-5,000.	+	+	+	+	+	+
Staph. pyog. aureus.	1-1,000.	+	+	+	+	+	+
	1-2,000.	+	+	+	+	+	+
	1-5,000.	+	+	+	+	+	+

(+ = Growth ; — = No Growth).

Growth was present in all tubes of anthrax spores and Staphylococcus aureus.

B. typhosus was killed in 5 minutes by 1-1,000, and in 12½ minutes by 1-2,000.

CYLLIN.

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).					
		2½	5	7½	10	12½	15
Anthrax spores.	1-1,000.	+	+	+	+	+	+
	1-2,000.	+	+	+	+	+	+
	1-2,500.	+	+	+	+	+	+
B. typhosus.	1-1,000.	+	+	+	+	+	+
	1-2,000.	+	+	+	+	+	+
	1-2,500.	+	+	+	+	+	+
Staph. pyog. aureus.	1-1,000.	+	+	+	+	+	+
	1-2,000.	+	+	+	+	+	+
	1-2,500.	+	+	+	+	+	+

(+ = Growth ; — = No Growth).

Growth was present in all the tubes.

In my second series of experiments, the procedure adopted by Klein in his experiments on *B. pestis* was followed. The same organisms were used as in my first series of experiments. The dilutions of the

(258)

disinfectants used in this series of experiments were the same as those employed by Klein in certain of his more crucial experiments. They were the same for formaline and phenol, as in my first series, but the dilutions of cyllin and corrosive sublimate were about twice the strength of those used in the first series. Agar cultures were used instead of bouillon cultures.

1. Emulsions of the different cultures were made in sterile distilled water, using one loopful of the culture in each case.
2. 5 c.c. of the different strengths of the disinfectant were put into sterile tubes.
3. To each tube of disinfectant was added .1 c.c. of the emulsion of the organism, and the mixture was then shaken thoroughly.
4. At end of intervals of 5, 10, and 15 minutes, 3 loopfuls of the mixture were smeared over the surface of an agar tube, and incubated for 8 days at 37° C. The following results were obtained.

FORMALINE (32 %).

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).		
		5	10	15
B. typhosus.	1-20.	+	—	—
	1-25.	+	+	+
	1-30.	+	+	+
Staph. pyog. aureus.	1-15.	+	—	—
	1-20.	+	+	—
	1-30.	+	+	+

(+ = Growth ; — = No Growth).

Growth was present in all tubes, except those of B. typhosus, where the dilutions were 1-20 and 1-25, and the exposures 10 and 15 minutes ; and Staph. pyog. aureus, where the dilutions were 1-15 and 1-20, and the exposures were 10 and 15 minutes.

CORROSIVE SUBLIMATE.

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).		
		5	10	15
Anthrax.	1-500.	+	—	—
	1-1,000.	+	—	—
	1-1,500.	+	+	+
B. typhosus.	1-500.	—	—	—
	1-1,000.	—	—	—
	1-1,500.	+	—	—
Staph. pyog. aureus.	1-500.	+	+	—
	1-1,000.	+	+	+
	1-1,500.	+	+	+

(+ = Growth; — = No Growth).

Growth was absent in dilutions of 1-500, after 10 and 15 minutes' exposure in the case of anthrax spores. With B. typhosus, growth was absent, after 5 minutes' exposure, in all except in the dilution 1-1,500. With Staphylococcus pyogenes, growth was present, after 15 minutes' exposure, in all tubes except dilution 1-500.

CYLLIN.

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).		
		5	10	15
B. typhosus.	1-500.	+	—	—
	1-600.	+	+	—
	1-700.	+	+	+
Staph. pyog. aureus.	1-500.	—	—	—
	1-600.	+	+	—
	1-700.	+	+	+

(+ = Growth; — = No Growth).

Growth was absent in dilutions of 1-500 and 1-600, after 10 and 15 minutes' exposure in the case of B. typhosus. With Staph. pyog. aureus, growth was absent after 5 minutes' exposure in dilution of 1-500, and absent after 15 minutes' exposure in dilution of 1-600.

IZAL.

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).		
		5	10	15
B. typhosus.	1-700.	—	—	—
	1-800.	+	+	—
	1-900.	+	+	+
Staph. pyog. aureus.	1-800.	—	—	—
	1-900.	+	—	—
	1-950.	+	+	—

(+ = Growth ; — = No Growth).

Growth was absent in all tubes of 1-700 after 5 minutes' exposure, and in 1-800 after 15 minutes' exposure, in the case of B. typhosus ; in the case of Staphylococcus, it was absent in 1-800 after 5 minutes' exposure, 1 in 900 after 10 minutes', and 1-950 after 15 minutes'.

SANITAS.

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).		
		5	10	15
B. typhosus.	Pure.	—	—	—
	1-5.	+	+	+
	1-10.	+	+	+
Staph. pyog. aureus.	Pure.	+	—	—
	1-5.	+	+	+
	1-10.	+	+	+

(+ = Growth ; — = No Growth).

Growth was present in all the tubes of B. typhosus and Staph. pyog. aureus, except the tubes in which the Sanitas was applied undiluted.

SAPO-CRESOL.

Organism.	Dilution of Disinfectant.	Time of Exposure (in minutes).		
		5	10	15
<i>B. typhosus</i> .	1-750.	+	—	—
	1-800.	+	+	—
	1-850.	+	+	+
<i>Staph. pyog. aureus</i> .	1-400.	—	—	—
	1-500.	+	+	—
	1-600.	+	+	+

(+ = Growth ; — = No Growth).

Growth was absent in dilution of 1-750 after 10 minutes' exposure, and in 1-800 after 15 minutes' exposure, in the case of *B. typhosus*; while in the *Staph. pyog. aureus*, it was absent after 5 minutes' exposure in dilution of 1-400, and, in 1-500 dilution, after 15 minutes' exposure.

CARBOLIC ACID CO-EFFICIENTS.

Disinfectant.	Sporing <i>B. anthracis</i> .	<i>B.</i> <i>typhosus</i> .	<i>Staph. pyog.</i> <i>aureus</i> .
Sanitas.	—	·025	·025
Formaline.	—	·25	·18
Cyllin.	—	6·0	5·0
Izal.	—	8·0	9·0
Sapo-Cresol.	—	9·37	6·25
Corrosive Sublimate.	16·6	25·0	6·25

The conclusions to be drawn from these two series of experiments on liquid disinfection—which are summarised in the accompanying table of co-efficients—appear to be as follows:—

1. *Formaline*.—In none of the dilutions used—though they are dilutions recommended for disinfectant purposes—and for the exposure tested, was formaline found to be completely efficient as a general disinfectant. The bacillus of typhoid was evidently, however, affected by it in the stronger dilutions. It was evident that even for non-sporing micro-organisms the strength of formaline solution should not be less

than 4 to 5 per cent., and for sporing organisms it ought to be still stronger. It appeared to have only about a fourth to a half of the germicidal power of phenol.

2. *Cyllin*.—In the degrees of dilution with which Klein obtained satisfactory results, and after the same exposures, cyllin in my hands has not yielded the same results. At the same time, it is evidently a disinfectant of great power, being effective against non-sporing organisms in dilutions of 1 in 500. My tests would lead me to rank it as about five to six times as potent as phenol.

3. *Izal* is another powerful disinfectant, being probably eight to nine times more active than phenol. Against some organisms, as *B. anthracis*, it is less active than corrosive sublimate, but against *Staphylococcus pyog. aureus* it is more active.

4. *Sapo-Cresol* is evidently a very active disinfectant. In my experiments, it was more powerful than izal towards *B. typhosus*, but less so towards *Staphylococcus*; while, in comparison with cyllin, it was more powerful against both these organisms.

5. *Sanitas* showed itself in these experiments to be by much the least active of the disinfectants tested. Apparently, it can only be relied upon when applied undiluted, and even then it may fail to kill sporing organisms.

6. *Corrosive Sublimate*, in the same dilutions as cyllin, showed itself to be more powerful than this antiseptic, especially in attacking the *B. typhosus* and anthrax spores. In its action on *Staphylococcus*, however, it is less effective than cyllin. This relative lack of efficiency against *Staphylococcus* is in agreement with the observations of Andrewes.

ON ECK'S FISTULA — OBSERVATIONS ON
FOUR DOGS, WITH A REVIEW OF THE
LITERATURE RELATING TO PREVIOUS
WORK ON THIS SUBJECT.

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On Eck's fistula.

THE great size of the liver in comparison with the other glands of the body, and the fact that all the blood from the gastro-intestinal tract must perfuse the liver before being poured into the systemic circulation, indicate that this organ must be of primary importance in the animal economy. The absolute exclusion of the liver from the circulation is impossible unless it be excised, an operation which is followed by such a degree of shock that any observations made on the animal afterwards are of little value, and are moreover soon cut short by its death. On the other hand, the partial removal of the liver from the circulation, by diverting the portal blood into the inferior vena cava, does not immediately affect the animal, so that it can be kept alive for some time and observations made on its metabolism. It is with some of the results of this latter operation on dogs that the present paper deals.

The first successful operation of this nature was performed on a dog in 1877 by Dr. von Eck (¹) of St. Petersburg. His object in doing the operation was to see whether it might not be possible, in this way, to relieve the congestion of the liver which occurs in cirrhosis of the liver. Of a total of eight dogs, only one survived the operation more than a few days, sepsis and thrombosis being the cause of death of the others. One dog, however, lived two-and-a-half months, and then escaped from the laboratory. No accurate observations were made on its metabolism. A few years later Stolnikow (²) also performed the operation successfully on dogs, but otherwise added nothing to Eck's findings.

Although, from these observations, no doubt could exist that the establishment of a porto-systemic fistula is not immediately fatal, it yet remained undecided whether there is any ultimate effect on the animal, and whether its metabolism is affected. To study these questions, Pawlow and Massen (³) performed the operation on a large series of dogs, taking every possible precaution to prevent sepsis, and they elaborated a

technique for sewing the veins together and making the opening between them, thus rendering the operation much less dangerous. Many of the dogs, thus operated on, lived several months, and an accurate study was made of their general condition and metabolism. In this part of the investigation M. Nencki and M. Hahn collaborated. The main results of these observations will be discussed later.

Following Pawlow and Massen's technique, several experimenters have performed the operation. The more important contributions have been by Magnanimiti ⁽⁴⁾, von Karltru ⁽⁵⁾, Sweet ⁽⁶⁾ and Herrick ⁽⁷⁾.

Three of von Karltru's dogs lived from one to nine months, and *post mortem* examination revealed the presence of an extensive collateral circulation around the liver, as a result of which much of the portal blood still circulated through this organ. Both Magnanimiti and von Karltru performed the operation, as recommended by Queirolo, in which the veins are made to anastomose below the vena pancreatico-duodenalis, which latter is tied. This operation, being on portions of the veins which are comparatively free, is much easier than Pawlow's, where the fistula is made close to the hilum of the liver, above the vena pancreatico-duodenalis. By ligating the pancreatico-duodenal vein, however, a collateral circulation to the liver becomes established by enlargement of the fine blood vessels in the ligamentum hepato-gastroduodenale, so that the object of the anastomosis is frustrated. Sweet employed a cautery wire, instead of Pawlow's scissors, in making the communication between the veins, and thus considerably simplified the technique. The vena pancreatico-duodenalis was not tied. His results were excellent.

In all these operations, it is most difficult to sew the veins together without very troublesome hæmorrhage. Dr. F. C. Herrick, working in this laboratory, has shown that the veins involved can be clamped a few minutes at a time without any permanent injury, and that while empty of blood they can be quickly and easily sewn together. The veins were anastomosed just below the opening of the pancreatico-duodenalis, which latter vein was not tied, so that the portal vein being ligated above it, its blood found free access to the vena cava. For making the communication between the veins he used a fine wire snare.

The Results of the Operation.—Apart from the general effect of so severe a surgical operation nothing unusual is commonly noted in the

dogs for some time, unless perhaps, as observed by Pawlow, they become irritable. The temperature immediately after the operation usually sinks a degree or so, to rise afterwards somewhat above the normal, at which it stands for about 10 days. The body weight may, or may not, gradually diminish. In about 10 days after the operation many of the dogs begin to show symptoms which are considered peculiar to the condition. At the outset these symptoms are variable in character. Some animals become very restless and excitable and tear and worry everything with which they come in contact, others become usually quiet and sleep most of the time. These conditions may alternate in the same dog. Sooner or later, more definite symptoms make their appearance; the animal (now lying down) becomes depressed and apathetic, and when he attempts to walk is found to be paretic in the hind legs so that he continually sits down. The gait is also distinctly ataxic, the legs being lifted unnecessarily high and then dashed on the ground, and, frequently, in awkward positions. If the limbs be placed in some unusual position—*e.g.*, crossed over one another—the dog leaves them there for some time. Pawlow considers this an important symptom and calls it catalepsy.

Supervening on these symptoms a gradual condition of hyper-excitability is next noted. The dog is continually moving about, but the movements are purposeless and in-coordinate. During this stage he becomes blind and anæsthetic, but retains consciousness and hearing. For example, one may push a needle into the pads of the paws and tramp on the toes without the dog taking the slightest notice, whereas, if the door of the room be opened, he will at once start up and try to make for it. That he is blind is shown by the fact that he does not attempt to avoid objects in his path but runs against them. Meanwhile, he is gradually becoming unconscious, and may now show convulsive movements of greater or less severity. The convulsive stage is followed by one of coma, in which the animal may die.

Many of the dogs recover from these symptoms, but, in such cases, can usually be easily caused to manifest them again by rough handling or excitement. In some cases the above symptoms are only partially seen, the first thing noted being convulsions. In others, the stages follow one another so quickly that they are indistinguishable as such.

From the outset, Pawlow, and others, noted that certain of the dogs

refused food almost entirely, in consequence of which they lost from 30-40 % of their body weight. Sooner or later, these dogs showed a very acute attack of symptoms which were usually fatal. Other animals ate sparingly, and were not affected so acutely. Some dogs, on the other hand, ate plentifully of certain foods and suffered only mildly. The ingestion of flesh was found to be the immediate cause of the onset of symptoms. Many dogs, having recovered from an attack, would afterwards refuse flesh and remain perfectly normal until again induced to take it, when another attack would result. Other dogs seemed from the very first to know instinctively that flesh was hurtful to them, and would absolutely refuse to eat it, and if offered no other food, starve instead.

Pawlow and his co-workers sum up their result regarding these observations by stating that "dogs in which the portal blood flows directly into the vena cava without passing the liver cannot stand flesh diet without the development of severe nervous symptoms which often terminate in death."

In the dogs which did not show symptoms, *post mortem* examination revealed a collateral circulation to the liver and a small opening between the veins. The presence of this collateral circulation in von Karltru's dogs undoubtedly explains the absence of the symptoms reported by him.

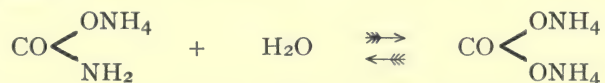
A careful chemical examination of the urine of these dogs was made by Nencki and Hahn. A relative increase in ammonia and uric acid, and a relative decrease in urea, were usually found. The urine was mostly alkaline in reaction. The most noteworthy abnormal change however, consisted in the presence of *carbamic acid*, and in greatest amount when the above symptoms were present. This led these workers to think that poisoning by carbamic acid might be the primary cause of the symptoms. To test this hypothesis, studies were next made of the pharmacological action of carbamic acid in dogs. When a five per cent. solution of carbamate of sodium in physiological saline was injected intravenously into normal dogs the following effects were noted. With 0.25 gram. carbamic acid per kilogram body weight the dog became drowsy and somewhat ataxic in his gait; with a dose of 0.3 gram. per kilogram the dog became more or less excited, sometimes excessively so; the movements were ataxic, and the sense of vision was lost, but hearing was normal. The skin was anæsthetic, and sometimes

a cataleptic state was noted. With 0.6 grm. per kilogram tonic and clonic convulsions were produced (simulating epilepsy), the saliva flowed from the mouth, the pupils were dilated. Anæsthesia and blindness were present. Usually, the dogs recovered. With larger doses tetanus with opisthotonus and cessation of respiratory movements, followed by death, were observed.

Exactly the same symptoms were produced in Eck's fistula dogs by placing carbamate of sodium in the stomach, the contents of which had previously been neutralized by giving sodium carbonate. On the other hand, no symptoms, except occasional vomiting, followed a gastric administration of carbamates to normal dogs.

From these interesting observations it seemed justifiable to conclude that the symptoms seen in Eck's fistula dogs are due to poisoning by carbamic acid. The liver is the (chief) site of urea formation, the immediate precursor of urea is carbamic acid, and large amounts of this substance are contained in the portal blood (being formed in the intestine, especially when flesh is being digested). Therefore, when the portal blood does not traverse the liver before entering the systemic circulation, the carbamic acid which it contains is retained somewhere in the organism until present in sufficient amount to induce poisoning.

Accepting these observations as correct, there would appear to be little doubt that carbamic intoxication is the cause of the symptoms, but if we consider the chemical nature of carbamic acid, and especially the extreme rapidity with which, in the presence of water, it becomes converted into ammonium carbonate, then it would appear that poisoning by *ammonium* is more probably the true explanation. When carbamate of ammonium comes in contact with water the following reaction ensues :—



The two arrows in this equation indicate that complete conversion of carbamate into ammonium carbonate does not occur, but, on account of reversible action, only until a certain equilibrium between carbamate and carbonate is established.*

* Haskins and I have recently shown that this decomposition of carbamate is extremely rapid, and that the relative amount of carbamate which remains undecomposed is relatively high.

Ammonium carbamate when dissolved in water will decompose until there is in the solution exactly the same amount of ammonium carbamate as if an equivalent amount of ammonium carbonate had been dissolved.

Consequently, as in the above experiments, when carbamate is injected intravenously, or when it is given by the stomach, it must have largely decomposed before gaining the blood. If carbamate is produced in the intestines after flesh has been ingested it will be largely converted into ammonium carbonate before getting far in the blood. In other words ammonium ions will have been produced from it.

Pawlow and Nencki did consider this probability, but thought that the symptoms produced by injection of carbamate-free ammonium salts were distinctly different from those which follow carbamate injection. Their comparison of the two (*i.e.*, ammonium and carbamate), however, was concerned only with the general symptoms, and these, being entirely objective, are necessarily difficult to compare. I have found that the effect on the mean arterial blood pressure and respirations of solutions of ammonium carbamate and ammonium bicarbonate are identical in every respect. Now, one of these will contain much more carbamate than the other, the bicarbonate containing only a small amount of carbamate, so that on the blood pressure at least the effect can be entirely ascribed to the ammonium ion.

We see then that the main question regarding these symptoms concerns not so much the presence of excess of carbamic acid in the organism as the presence of an excess of ammonia. Even if the symptoms *are* due to carbamate intoxication there must be excess of ammonia. In their more recent publications the St. Petersburg workers have accordingly confined their attention to the behaviour of the *ammonia content* of the organism in Eck's fistula dogs. At the outset, in doing this, it soon became evident that exact information regarding the quantitative distribution and the source of ammonia in the *normal* animal body had first of all to be obtained.

Nencki, Pawlow and Zaleski ⁽⁹⁾ and later Salaskin ⁽¹⁰⁾, by the use of accurate methods for the determination of ammonia in the blood and the various organs, have recently furnished this information. The following are among their main conclusions.

In the arterial blood of dogs fed on flesh the ammonia content is about 1.5 mg. per 100 cc. It is very constant.

In the portal blood, under the same conditions, from 3 to 4 times as much ammonia is present, being, however, variable in amount. In the blood of the branches of the portal vein, coming from the stomach, pancreas and intestines, the percentage of ammonia is still higher.

In the mucosa of the stomach, the brain and lungs a very high ammonia percentage exists.

During fasting, the percentage of ammonia in all the above situations falls markedly (0.4 mg. per 100 cc. in arterial blood).

Concerning the source of the ammonia in the portal blood a large part of it comes from glandular activity, for it appears in the portal blood when a fasting dog is merely tempted with savoury food ("sham feeding.") When food is ingested, still more ammonia appears. Its source is therefore double—from the active glands and from the food. Ammonia is produced elsewhere in the body from the katabolism of proteid food stuffs.

The above observers have also examined the ammonia content of the blood and various organs in *Eck's fistula dogs*, and have come to the following conclusions.

In the blood and urine there may or may not be an increased amount of ammonia, but in dogs which have died of the above symptoms a large excess of ammonia is always found in the brain.* Salaskin sums up the main conclusions of this part of the investigations of the St. Petersburg workers in the following manner.

After the establishment of the fistula, when the dog is on a flesh diet, the ammonia which is produced by the activity of the gastro-intestinal glands and from the food is carried by the portal blood directly to the systemic circulation. Under normal conditions by far the greater proportion of this ammonia will become converted into urea in the liver. In the circulation the ammonia produced in the tissues by katabolic processes is added to it. A part of this excess of ammonia in the blood is filtered through the kidneys into the urine, some passes *via* the hepatic artery to the liver, and is converted into urea† and the remaining excess becomes stored away in some unknown combination in the organs, especially in

* A table giving all the details of these observations will be found in Salaskin's paper.

† Some may also be converted in urea elsewhere than in the liver.

the brain. When a certain amount of ammonia has accumulated in the brain, symptoms appear, and if the dog recover from these and receive no more strongly nitrogenous food for some time, this excess of ammonia in the brain will gradually be carried away in the blood again, and either become converted into urea or escape into the urine. It will be evident from the above that there may or may not be excess of ammonia in the blood and urine when symptoms are present.

To induce the above symptoms more acutely, Nencki and Pawlow (11), after making an Eck's fistula in a dog, removed as much ($5/6$ to $7/8$) of the liver as possible. The dogs lived on an average from two to three hours after the operation. One of the animals remained in a comatose state after the removal. One dog, however, so far recovered as to run about for some time, but soon showed tetanic convulsions ending in death. The symptoms were not strictly like those portrayed above. In two of these dogs the ammonia in the blood and urine was found to be increased. The urea in the urine was diminished, whereas the urea in the blood remained normal in amount. This last result is due to the fact that urea formation is not confined to the liver but may occur elsewhere in the organism. The increase of ammonia in the blood was not great enough to account for death. It was consequently thought that perhaps some toxic substances had been produced by the removal of the liver. No experimental evidence of this could, however, be obtained by inoculating a normal rabbit with some of the urine excreted by the dogs.

In other dogs with Eck's fistula instead of removing the liver, the hepatic artery was ligated. Some of these dogs lived 12 to 15 hours, apparently recovering well from the operation, but afterwards passing into a comatose condition. In one of these dogs, the blood was examined chemically. For about seven hours after the operation this dog did not show anything abnormal, but then clonic convulsions appeared, passing into coma, and ending in death ($8\frac{1}{2}$ hours after operation).

The ammonia content of the blood was found to be unaffected by this operation, so that death must have been due to some cause other than ammonia intoxication.

These observations recall others by Lieblein (12) in which the liver cells were destroyed by injecting acid into the hepatic ducts. The dogs became comatous, and died much in the same way as after removal of the

liver. Something other than ammonia must have been the cause of death.

It would occupy too much space to discuss the observations of Magnanini and von Karltreu here. They are rendered valueless in this connection by a collateral circulation which developed around the liver.

I have given a fairly extensive review of the work of the St. Petersburg observers in this direction, as I believe that, although widely known in a general way, the exact details of this most interesting experimental *disease* are little known. With the recent simplification by Sweet and Herrick in the technique of Eck's operation, more extensive observations of the symptoms are rendered possible, and the question as to whether carbamate or ammonium poisoning is their cause, or whether they are not due to some other poisonous body (toxin), should not long remain an open one.

The behaviour of several of the dogs operated on by Sweet was observed by P. B. Hawk (¹³). Only mild, transient symptoms were noted when flesh powder was given, but they were much more severe when meat extract or fresh flesh was also given.

It was further noted that the injection of sodium carbamate did not produce any symptoms similar to those observed in dogs with Eck's fistula, either when the salt was given to normal or to Eck fistula dogs.

Rothberger and Winterberg (¹⁴) have recently communicated a series of very accurate observations on twenty-two dogs in which Eck's operation had been performed. They conclude that the symptoms are probably not due to carbamate intoxication, but rather to some other toxic substance which the liver normally destroys. In this connection they point out the extreme importance of the liver in antagonising the action of many poisons (*e.g.*, strychnine), when these are absorbed into the portal blood from the intestine. In comparing the symptoms, presented by Eck fistula dogs, with those induced by administration of carbamate or substances which would produce it, they found very considerable differences.

I shall in this connection offer a brief account of some observations on the general condition of four Eck's fistula dogs operated on by Dr. F. C. Herrick. I shall then give the results of a chemical examination of the urine of three of these animals. Unfortunately, the blood and organs were not examined for their ammonia content.

Regarding the condition of the dogs after the establishment of the fistula, I have noted undoubted nervous symptoms in two of them (Nos. 2 and 4), but no definite symptoms in the other two (Nos. 1 and 3). The following is an account of the behaviour of the dogs:—

Dog 1.—Female, weighing 25 lbs. The operation was performed on January 21st, 1904. For six days after it no food was taken, there being slight fever. From January 28th, to February 12th (15 days) a diet of 300 gr. bread and 50 gr. butter was taken, and during this period the dog increased in weight from 21 to 23 lbs. On two occasions (*viz.*, on the 4th and 9th of February) meat extract was added to this diet. A period of six days' fasting followed, and then a bread and butter period of eight days, after which flesh was given in gradually increasing amounts (see Table I.).

At no stage were any phenomena noted like those described by Pawlow and Massen, but marked *nephritis* with accompanying symptoms set in, which at one stage looked as if they would prove fatal. On this account it may be of interest to describe the history more in detail.

From the very outset albuminuria was observed. Until the day after the first administration of meat extract, however, the proteid was present only in traces, but on this day it increased distinctly in amount, the increase continuing daily until, after a few days, about 50% of the total nitrogen excreted was present as proteid. Fatty, cellular and hyaline casts were also abundantly present in the urine, which also contained globular bodies soluble in ether. The high excretion of nitrogen occurring even when no food was given is accounted for by the presence of proteid. The animal became very much emaciated. Œdema of the abdominal wall was very marked, and an interstitial keratitis developed. On account of the presence of so much proteid, the quantitative estimations of the various urinary constituents were rendered very difficult, and had to be discontinued because of their inaccuracy. The dog was carefully treated, being fed for some time largely on milk; and her condition gradually improved, the proteid diminishing slowly till it had almost disappeared. After two months she was removed to the country where she became perfectly well. After four months she was killed, and a *post mortem* examination made. It was found that the ligature had been applied above the vena pancreatico-duodenalis, and that the opening between the vena cava and vena porta had closed. Nothing abnormal

was found in the kidneys. A microscopical examination was unfortunately not made.

Dog 2.—Male, about 40 lbs. weight. Operated March 8th, 1904. He recovered well from the operation, and in three days was placed on a bread and milk diet which was changed to one of flesh (200 gr.) three days later. For six days the dog took this eagerly, but on the seventh refused it. During this day he was somewhat restless. Next morning he was found lying on his side in an unconscious condition, with the head thrown back and the hind limbs drawn up on the abdomen. The head was constantly moved backward and forward on an average of about 70 times per minute, but this rhythm was irregular. Each time the head was drawn back the eyelids closed. On pricking the skin with a needle no reflex movement resulted; on placing the limbs in an unusual position they were not withdrawn (Pawlow's catalepsy). Conjunctival reflex normal; rectal temperature 39° C.; respirations 12-14 per minute; pulse 110-120 per minute, and irregular both in force and rhythm.

In an hour or so the clonic contractions of the head disappeared, and after about three hours the dog seemed to have recovered and took some milk. He was kept on a bread and milk diet for some weeks and gained weight. Flesh was then given him again, but no further symptoms were ever noted. He lived several months. An opening, measuring one centimeter across, was found between the veins, and no collateral circulation.

Dog 3.—Male, about 60 lbs. There was a good recovery from the operation. Nothing unusual in the dog's behaviour was noted even although he took large quantities of flesh. Three weeks after the first operation a second operation was performed, when it was found that a large vein entered the portal above the vena pancreaticoduodenalis. A second ligature was therefore applied to the portal vein quite close to the liver. Three days after this second operation the dog was again placed in his metabolism cage, and the urinary analysis proceeded with (see Table II.). This dog did not show any definite symptoms, and died from pneumonia three weeks after the second operation. He rapidly lost in weight, and was usually feverish, some sepsis having probably been developed.

Dog 4.—Operated February 27th, 1905. Food was withheld until March 8th, when a diet of milk, bread and butter was given, and

continued till March 23rd. While on this diet the dog slowly lost weight (from 28 lbs., to 25 lbs. in 26 days), but appeared normal in every other respect. Though very quiet while we were in the laboratory, she was extremely restless during the night time, for on several occasions she tore her way out of the strongly made metabolism cage in which we kept her, on one occasion by gnawing through a plank of wood, and on another by tearing away the stout wire netting on the lid of the cage.

On March 23rd, the daily diet was changed to one of 400 gr. flesh. At first, she eagerly ate all the flesh, but in a few days began to take only a portion of it. Otherwise she appeared quite normal till April 7th, when it was noticed that she looked uncomfortable, and would not lie quietly in one place as had previously been her custom, but wandered aimlessly about the laboratory. On the morning of April 8th, this restlessness was very marked. At 11:30 a.m., on this day, her gait was seen to be ataxic, and at 12 noon she became paretic, particularly in the hind limbs, so that she could no longer stand, but lay on her side. At this time, her respirations were, on an average, 48 per minute, sometimes becoming rapid and gasping; pulse 140 per minute, regular; rectal temperature 39.5°C .; there was very profuse salivation; the ears showed fine tremors; the pupils were very contracted and did not react to light; on pricking with a needle between the pads of a paw, or on nipping with a strong forceps, no reflex movements resulted (skin apparently anæsthetic); the limbs were extended and the muscles rigid, especially those of the hind limbs; when a limb was placed in an unusual position it was kept there for some considerable time (Pawlow's catalepsy). Some of the saliva was collected and found to be distinctly alkaline in reaction; it did not give any reaction for ammonia when boiled with alkali. From time to time she made futile attempts to assume the erect posture, and in these attempts moved the fore limbs as in swimming. She passed urine, which was collected and found to be alkaline in reaction, with 0.2 % of ammonia, 71 mm. CO_2 and 28 mm. carbamate CO_2 .

At 3:15 p.m. the head and neck were found to be rigidly thrown back almost at right angles to the spinal column (opisthotonus); the muscles of the hind limbs were now relaxed, and those of the fore limbs very rigid; pulse 160 and of low tension; respirations slower (26)

but regular and deep; the pupils still very contracted. She now lay quietly on her side and made no attempts to assume the erect posture.

For the next $2\frac{1}{2}$ hours very little change was noted in her condition, except that the respirations became laboured and the pulse of lower tension; the rectal temperature fell to 38.5° C.; the extremities became cold to the hand. At 5:48 p.m. she was wrapped in cloths to keep her warm, and in moving her for this purpose, convulsions were induced, in which the head and neck were thrown far back on the body and the fore limbs stiffly extended. The respirations became very rapid and gasping, and the pulse almost imperceptible. This convulsion lasted 7 minutes, and as she had then become quiet, she was left till next morning, when she was found dead, lying in the same position as when last seen. Autopsy revealed the existence of a perfect Eck's fistula without any collateral circulation.

It will be seen that the dogs observed by us behaved in general exactly like those of the St. Petersburg workers, and that a flesh diet seemed to bring on the symptoms. The marked nephritis, which was noted in dog 1, was no doubt due to disturbance in the kidney circulation, the exciting cause perhaps having been the excess of meat extractives given the animal on the day before the albuminuria became marked.

The Chemical Examination of the Urine.

This was carried out in the case of three of the above dogs (Nos. 1, 3 and 4).

Nacki and Hahn (*vide* page 270) have shown that in the urine of Eck's fistula dogs there is a relative increase in the excretion of ammonia and of uric acid, and relative decrease in that of urea. They also found carbamates in the urine. In our investigation we therefore examined total-nitrogen (Kjeldahl-Gunning method); urea-nitrogen (Mörner and Sjöqvist and Folin's method); purin-nitrogen (Camerer-Arnstein method) and ammonia-nitrogen (Folin's method). We likewise tested for the presence of carbamates by the Abel-Drechsel method, and later, by the method elaborated by us and described elsewhere.*

* Macleod and Haskins: The Quantitative Estimation of Carbamates. American Journal of Physiol., 1905, xiii., p. 17.

Dog 1 (Table I.). As already pointed out, this dog developed a marked nephritis, so that the urinary analysis was rendered extremely difficult and possibly inaccurate towards the end of the investigation.

Until proteid made its appearance in any considerable quantity the relative amount of urea and ammonia were about the normal. The purin-nitrogen excretion previous to the day on which meat extract was given averaged about 0.04 gr., which is distinctly higher than the average for a dog of this size. When meat extract was added to the otherwise purin-free diet, a very distinct increase in purin excretion resulted.*

After albuminuria developed, the relative amount of urea-nitrogen steadily fell, more than 50% of the total nitrogen being excreted in the form of proteid. The absolute amounts of urea and ammonia remained, however, nearly unchanged.

Urine of Dog 3.—The results of this investigation are given in Table II., from an examination of which it will be seen that the daily excretions of nitrogen, ammonia and urea were very high and to a corresponding degree, the nitrogen of ammonia being on an average about 7 per cent. of the total nitrogen, and that urea about 79 per cent. The rapid emaciation of this dog probably accounts for the high nitrogen content of the urine.

On March 15th a dose of 10 grams of ammonium acetate was given by the stomach tube with the result that on the same day and the day following a marked diuresis was induced, but with no increase of the ammonia excretion on either day, whereas on the day following there was a very marked increase in the excretion of urea, its nitrogen being 88 per cent. of the total nitrogen. On the 17th of March 5 grams of ammonium carbonate (containing, therefore, carbamate) had likewise no effect on the ammonia excretion, but raised very considerably that of urea.

Regarding the purin excretion, it will be seen that this was fairly constant (viz., about 0.04 gms. purin-nitrogen), except on the 17th of

* Burian and Schur ("Über die Stellung der Purinkörper im menschlichen Stoffwechsel," Arch. f. d. ges. Physiologie, 1901. Bd. 87, p. 291.) have shown that in normal dogs about 5% of ingested oxypurin (xanthin and hypoxanthin) reappear in the urine. Our object in giving the meat extract in the above experiment was to see whether the same *integral factor* would hold for a dog with Eck's fistula. The incidence of albuminuria, however, renders the observed result too inaccurate to draw any conclusion.

March when it was considerably higher. This was the day on which ammonium carbonate was given, and on which there was a marked diuresis. The result agrees with those of Burian and Schur * on normal dogs, and may possibly be explained in the same way as they explain theirs; that the circulation through the kidneys had been rendered more rapid so that more purin was excreted into the urine before it had time to be destroyed in the liver and other purin-destroying organs.

It is further of interest to note that the purin excretion was not much higher than normal.

In dogs weighing about twenty pounds the average daily excretion of purin-nitrogen was found by Burian and Schur to be about 0.035 gr. Omitting No. 7 in our table, the average for the Eck's fistula dog is 0.039 gr. In Burian and Schur's dogs a flesh diet was given, in ours a variable diet. The figures are therefore not strictly comparable.

Nencki and Hahn described the uric acid excretion in dogs with Eck's fistula as distinctly higher than normal. This finding we have therefore been unable to confirm.

At no period during the investigation was there ever, in this dog's urine, the slightest trace of carbamate; the urine was acid or amphoteric throughout, so that no noteworthy amount of carbonic acid gas was present. Nevertheless, the dog had been fed with meat, and the *post mortem* examination had revealed a perfect Eck's fistula. Not even when given ammonium carbonate solution (which contains carbamate) did any carbamate appear in the urine.

Indeed, the urine of this dog, as a result of the chemical analysis, must be regarded as perfectly normal, the relation both of urea and of ammonia to total nitrogen being, for a meat diet, about the normal one.

In Dog No. 4, weighing $12\frac{1}{2}$ Kg., the urinary analysis was not so complete, but nevertheless a catheter specimen was daily examined for total CO_2 and carbamate CO_2 . The difficulty in making a complete analysis lay in the fact that it was impossible to keep this dog confined in her cage (see p. 278).

While on a diet of bread and milk, the urine was frequently alkaline in reaction, and, when so, was found to contain a considerable amount of carbonic acid and carbamate. The carbamate CO_2 did not, however, bear any constant proportion to the total CO_2 . The alkaline urine of

* Burian and Schur: *Loc. cit.*, s. 342.

TABLE

Date.	No.	Dog's Weight.	Food.				Urine—24 Hours.					
			Flesh in gms.	Bread in gms.	Butter (gms.).	Total N (gms.).	Amount in c.c.	Specific gravity.	Reaction to litmus.	Total nitrogen (gms.).	Urea N (gms.).	Purin N (gms.).
Jan. 21	No food.		
Jan. 23
Jan. 24
Jan. 25	1
Jan. 26
Jan. 27	2
Jan. 29	3	182	40·2	2·229	370	+	Acid	3·70	...	0·0185
Jan. 30	200	40	2·450
Jan. 31	250	40	3·062
Feb. 1	4	150	30	1·837	900	...	Acid	1·968	1·419	0·0266
Feb. 2	5	21 lbs.	...	300	40	3·675	500	...	Alkaline	1·904	1·428	0·0577
Feb. 3	6	300	50	3·675	400	...	Neutral	3·304	2·576	0·0451
Feb. 4	7	300	50	3·675	350	...	Acid	2·156	1·660	0·0495
Feb. 5	300	50	3·675
Feb. 6	8	22 lbs.	...	300	50	3·675	750	...	Acid	+	1·606	0·072
Feb. 7	300	50	3·675
Feb. 8	9	300	50	3·675	700	...	Acid	3·535	2·825	{ 0·0789 0·1067
Feb. 9	10	300	50	3·675	600	...	Acid	3·629	+	0·2142
Feb. 10	300	50	3·675
Feb. 11	11	23 lbs.	...	300	50	3·675	650	...	Acid	4·650	3·1213	*0·0842
Feb. 12	12	230	45	2·817	400	...	Acid	+	+	+
Feb. 13	13	24 lbs.	...	0	0	0	350	...	Acid	2·974	1·700	+
Feb. 14	0	0	0
Feb. 15	14	23 lbs.	...	0	0	0	600	...	Acid	2·528	1·394	+
Feb. 16	0	0	0
Feb. 17	...	22 lbs.	...	0	0	0
Feb. 18	16	21½ lbs.	...	25*	0	...	600	...	Acid	4·032	+	+
Feb. 19	17	21 lbs.	...	75	0	·918	400	...	Acid	3·450	1·848†	...
Feb. 20	18	21½ lbs.	...	100	0	1·225	some lost.	1·783%	0·722%	...
Feb. 21	100	...	1·225
Feb. 22	100	...	1·225
Feb. 23	19	21½ lbs.	...	100	...	1·225	500	...	Acid	3·556	2·441	...
Feb. 24	20	100	...	1·225	355	...	Acid	3·538	+	...
Feb. 25	...	21½ lbs.	...	100	...	1·225
Feb. 26	21	21½ lbs.	200	6·8	550	...	Acid	3·591
Feb. 27	22	21 lbs.	200	6·8	400	...	Acid	6·451
Feb. 28	23	...	200	6·8	500	...	Acid	6·552
Feb. 29	24	20 lbs.	400	13·6	400	...	Neutral	7·481

I.

Urine— 24 hours.	Carbamic acid.	NH ₃ N to total N.	Urea N to total N.	Abnormal substances.	Remarks.
NH ₃ N (gms.).				Albumin.	
...	Dog (female) Eck's fistula established. Came out of anæsthetic well.
...	Dog normal—Falck's operation, no food, no water.
...	Unsuccessful catheterization under ether with no food.
...	Catheter passed 12:15 — some urine lost in struggling—NH ₃ doubtful.
...	Starved—wasting.
...	Catheter 12:15—some blood mixed with urine.
0'102	Very little urine in basin eight days after operation.
0'0703	nil.	..	1:1'39	...	Each dog's urine placed on ice—copious washing.
0'1078	trace.	1:17'6	1:1'33	...	
0'1097	...	1:30'1	1:1'28	...	
0'0798	..	1:27	1:1'299	...	10 g. gr. beef extract given after catheterizing.
0'372	+ +	...	High purins.
0'153	trace.	1:23'1	1:1'24	...	One of the purin precipitates with AgNO ₃ , inaccurate.
0'2688	...	1:13'1	+ +	0'5% (Esbach).	20 gms. beef extract given after catheterizing.
0'1483 +	...	1:21	1:1'49	{ Up to U mark in Esbach.	*High purins and calculation only approximate— proteids separated by boiling in acid.
(0'113)	...	1:17'1	1:1'74	{ 13mm. below mark U.	
0'174	...	1:33'2	1:1'81	{ 9mm. below mark U.	
0'076	Hyalin, fatty and cellular casts in urine; nucleo- proteid also present.
...	Sediment—casts, bladder cells. Globular bodies like leucin.
0'537	...	1:75	{ *Given 2 hours before catheterization. Very faint ppt. with HA.
0'048	..	1:72	1:1'86	...	
...	1:2'46	...	The globular bodies dissolved in ether, not in hot water, therefore, fat.
0'0858	...	1:41'4	1:1'45	At mark U.	(†Only one urea estimation).
0'1215	...	1:29	...	7%	
0'1355	...	1:26'5	...	7%	
0'1635	...	1:39'4	
0'1736	5%	
0'1344	7%	

the 20th of March was slowly evaporated almost to dryness, and then made up with distilled water to the original bulk; the resulting solution was found still to be alkaline in reaction, showing that the original alkalinity of the urine had not been due to ammonia. On March 11th, however, the ammonia excretion was considerably above the normal, but became normal again two days later.

While on meat diet, the urine was unfortunately not examined until the day on which nervous symptoms appeared, when an alkaline urine was found to contain both CO_2 and carbamate CO_2 .

It would appear from this last analysis as if there were some relationship between the presence of carbamate and the nervous symptoms, but if, on the other hand, we bear in mind that it was while on bread and milk diet that this carbamate was found—*i.e.*, when the urine was alkaline—then we can explain the presence of carbamates as due to the interaction of soluble carbonate in the urine with the ammonia salts. Haskins and I have shown that whenever the urine contains soluble carbonates (*e.g.*, after citrate ingestion, or when it is allowed to stand a few days) carbamate is formed in this way.*

So far as our chemical investigation of the urine went, we could not detect anything abnormal in either of the three dogs, except the remarkable albuminuria developed by the first dog.

Our results were in general confirmatory of those of Pawlow, Hahn, Nencki and Massen, and point to the fact that in future investigations into the cause of the peculiar nervous symptoms described above, urinary analysis alone will not suffice, but must be supplemented or even replaced by careful analysis of the blood and organs.

To Dr. H. D. Haskins the author owes a deep great debt of gratitude for the valuable assistance which he rendered in conducting the chemical analysis. To Dr. F. C. Herrick also the author's thanks are due for the use of the dogs on which he so successfully performed Eck's operation.

* Macleod and Haskins, *Loc. cit.*, p. 279.

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ON THE ACTION OF CERTAIN BACTERIA IN
PRODUCING CELL-NECROSIS, WITH SPECIAL
REFERENCE TO THOSE OF THE BACILLUS
ENTERITIDIS (GÆRTNER) GROUP.

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On the Action of Certain Bacteria

In producing Cell-necrosis, with Special Reference to those of the
Bacillus Enteritidis (Gärtner) Group.

IT is well recognised that certain bacteria have the property of inducing a necrotic action in the cells attacked by them. These organisms may be roughly classed under two heads. There are, first, those which cause lesions more or less limited to one tissue or organ, for example, *B. diphtheriæ*, *B. mallei*, *B. necrophorus* and *B. typhosus*. The necrotic areas occurring in the liver in typhoid in man may be cited as an instance in which these particular lesions occupy a secondary place in the pathology of the disease. There is, however, another group of organisms, including *B. pseudo-tuberculosis rodentium*, *B. pestis* and the tubercle bacillus, which cause widespread focal necroses, presenting a characteristic pathological picture.

The necrotic effects are generally attributed to bodies—proteins of Buchner—in the cell-substance of the bacteria. In regard to the nature of these bodies, however, very little is so far known.

It is proposed in what follows to record some observations of a guinea-pig disease, recently met with in Bombay, due to an organism belonging to the *B. enteritidis* (Gärtner) group, with lesions closely resembling those of pseudo-tubercle. The attempt will also be made to correlate its pathological features in respect of the characteristic necroses with those of diseases caused by other members of the group.

In the Plague Research Laboratory, a considerable number of young guinea-pigs in the stock died apparently from a common disease during the monsoon months, *i.e.*, June, July, August and September. The animals were about three or four weeks old; the disease was not observed among full-grown guinea-pigs. The *post mortem* appearances of several of those examined are appended.

Guinea-pig 1 :—examined 27/7/05.*Naked-eye appearances—*

No enlarged glands; liver studded with fine nodules; spleen enlarged and congested—no nodules seen. Small intestines markedly congested. Kidneys show minute punctate hæmorrhages under the capsule. Lungs somewhat congested.

Microscopical examination—

Spleen smear shows bipolar organisms resembling *B. pseudotuberculosis*; many of these are in the cells. Heart-blood contains fairly numerous bipolar organisms, some being in the mononuclear cells.

Animal experiment—

The spleen and heart-blood were rubbed into a shaved area of skin of a guinea-pig which died of hæmorrhage from the lungs (probably traumatic) within 24 hours. No organisms were seen in the heart-blood of this animal, but a few bipolar organisms were observed in a spleen smear. There was a cutaneous reaction at the site of infection, and the inguinal glands were enlarged.

Cultural test—

An organism was isolated from the heart-blood which corresponded culturally, morphologically, and in certain sugar reactions with *B. enteritidis* (Gærtner).

Guinea-pig 2 :—examined 31/8/05.*Naked-eye appearances—*

Subcutaneous œdema; no enlarged glands; spleen very dark in colour—markedly enlarged and studded with grey nodules exactly resembling those of pseudo-tubercle. Very fine nodules were present in the liver. Small intestines extremely congested. A small amount of pleural and peritoneal effusion; lungs apparently normal.

Microscopical examination—

Heart-blood—very few bipolar organisms.

Spleen and Omentum—very numerous bipolar organisms.

Cultural test—

An organism similar to that from guinea-pig 1 was isolated from the heart-blood.

Guinea-pig 3:—examined 5/9/05.*Naked-eye appearances—*

Spleen enlarged and studded with grey nodules. Mesenteric glands enlarged and congested. Hæmorrhages in the large intestine. Suprarenals were congested and showed small hæmorrhages.

Microscopical examination—

Heart-blood—no organisms seen.

Spleen—organisms, very like those of pseudo-tubercle, fairly numerous.

Culture from heart-blood gave similar colonies to those previously obtained.

Guinea-pig 4:—examined 6/9/05.*Naked-eye appearances—*

Spleen much enlarged, with yellow and grey nodules—a few also in the liver. Small intestines congested. Lungs very congested.

Microscopical examination—

Spleen—few, if any, organisms seen.

Heart-blood—nothing definite.

Culture—Same organism as above obtained from spleen.

Animal test—

The spleen was rubbed into a shaved area of the abdomen of an adult healthy guinea-pig weighing 520 grammes. On the third day the inguinal glands could be felt to be enlarged. It steadily lost weight throughout, the last weight, on the 10th day, being 400 grammes. The animal was then killed by chloroform, and the following appearances were noted:—

Naked-eye.—Extensive purulent ulceration of the skin limited to the site of inoculation and burrowing into the subcutaneous tissue. Double inguinal buboes, the surrounding blood-vessels being moderately injected.

Spleen—somewhat enlarged with some yellow and white nodules; a few similar modules in the liver. Small intestines very hyperæmic.

Microscopically.—No organisms were seen in the buboes or organs.

Cultures.—The pus from the cutaneous reaction furnished a pure culture of the organism. The heart-blood proved sterile.

Guinea-pig 5:—examined 15/9/05.*Naked-eye appearances—*

General subcutaneous congestion; no enlarged glands. Spleen enlarged and densely studded with grey necrotic foci; there were very few in the liver also. The lungs contained a few grey nodules, each surrounded by a ring of congested lung tissue. The stomach and small intestines were extremely congested.

Microscopical examination—

Spleen—Fairly numerous deeply stained bipolar bacilli.

Heart-blood—a very few similar organisms.

Cultures—

Heart-blood—pure colonies of the organism.

Liver—pure colonies of the organism.

Spleen—pure colonies of the organism.

Guinea-pig 6:—examined 15/9/05.*Naked-eye appearances—*

Inguinal glands slightly enlarged.

Spleen—somewhat enlarged.

Liver—a few very small nodules.

Left suprarenal capsule showed nodules, and on section was seen to be almost wholly converted into cheesy masses. Lungs extremely congested. Stomach and intestines apparently normal.

Microscopical examination—

Nothing definite seen in heart-blood or in the organs. Suprarenal capsule showed fairly numerous bipolar bacilli arranged in pairs.

Cultures—(from heart-blood) furnished the usual growth.

It will readily be appreciated from the details given above how markedly the lesions simulate those met with in the well-known pseudo-tubercle of guinea-pigs. The characters of the bacillus isolated from the organs may be described briefly as corresponding closely to those of *B. enteritidis* (Gærtner). Culturally, and in its morphology, it gave evidence of being not far removed from *B. coli*. It can, however, be readily differentiated from the colon bacillus by the fact that it does not ferment lactose. It also fails to split up cane-sugar, but produces

acid and gas in media containing dulcitol. Attention may be drawn to the animal experiments in the case of guinea-pigs 1 and 4. It would appear that a cutaneous infection can take place by using the method of Albrecht and Ghon. In the case of guinea-pig 6, a chronic form of the disease was produced, although it is possible that by varying the experimental conditions, *e.g.*, by raising the virulence of the bacillus artificially, an acute condition, resulting fatally in a few days, might be brought about. Unfortunately, lack of time from pressure of other work rendered it impossible to carry out further experiments in this direction. The significance of the cutaneous method of infection will be referred to later.

It may appear somewhat curious that, although the disease shows well-marked lesions, very little can be found concerning it on consulting the literature. Nocard and Lécainche in their classical work on the microbic diseases of animals (edition 1903) make no mention of it. MacConkey (¹), in a foot-note to a recent paper dealing with lactose-fermenting organisms, gives a brief description of its main features. He notes that an organism indistinguishable from *B. enteritidis* (Gærtner) seemed to be the cause of a small epidemic amongst the experimental guinea-pigs at the Serum Institute of the Lister Institute near London, and refers to the resemblances of the naked-eye appearances to those of pseudo-tuberculosis rodentium.

Kovářzik (²) has given an account of an epizootic, amongst guinea-pigs, which he ascribed to a variety of *B. coli*. Lehmann and Neumann, however, include the bacillus in the *B. enteritidis* (Gærtner) group. The principal points of his paper may be briefly summarised. *Post mortem*—there were found greyish-white necrotic foci on the surface of the liver and spleen. In smears of these organs the bacilli were isolated, but sometimes occurred in pairs—short rods (Gram-negative) with rounded ends. Coccal forms occurring in cultures; giving on agar, greyish-white glancing colonies, bluish by transmitted light, later white; gelatine not liquefied; acid and gas in glucose; no clotting of milk nor indol reaction. Cultures were very pathogenic to guinea-pigs, especially when inoculated intraperitoneally—an infection was also produced through the intact conjunctiva. It was pathogenic to rabbits by intravenous but not by subcutaneous inoculation, and killed also by injection into the large intestine. Gray rats were killed by feeding on cultures—

—pigeons by intramuscular injections. Kovářík considers that his bacillus is most nearly related to Lochmann's *B. caseolyticus*.

Lochmann (³) has described a bacillus which, he believes, is closely related to *B. coli*, and which he isolated from 4 guinea-pigs dead of experimental tuberculosis. Culturally and morphologically it resembled *B. coli*. It differed, however, from the typical colon bacillus in the production of alkalinity in milk, by the absence of the indol reaction and by its pathogenicity for animals by feeding. Greyish-white deposits were frequently observed in the liver and spleen of experimental animals. It is very probable that Lochmann's bacillus is identical with those referred to above, and that it would be more accurate to place it in the *B. enteritidis* group, and indeed Lehmann and Neumann, in their handbook, have adopted this course.

Before proceeding to discuss the lesions produced by other members of the group it may be of interest to record a few cases in which a similar organism was encountered during the ordinary routine work of the laboratory.

I.—*Mus decumanus* brought to the laboratory dead.

From the heart-blood of a *mus decumanus* brought dead to the laboratory for examination, a culture was obtained of two kinds of colonies. Subcultures of the predominating colonies yielded a bacillus corresponding in all the tests applied to the *B. enteritidis*. The *post mortem* examination revealed evidences of an acute disease, the spleen being very large, soft and deeply congested; the peripheral glands were enlarged; the serous membranes were injected, and there were hæmorrhages in the epicardium. There were signs of commencing putrefaction in the organs. No animal test was performed.

II.—*Mus decumanus* brought to the laboratory dead.

Naked-eye appearances—

Nothing pathological was seen, except that the lungs were congested, and showed hæmorrhages. The abdominal organs showed signs of putrefaction.

Microscopically—

Nothing definite was seen in smears.

Cultures of the heart-blood on agar gave numerous grey, circular smooth colonies which, on detailed examination, proved to be of the type of *B. enteritidis*.

Animal test—

The spleen of this rat was rubbed into the shaved belly of a guinea-pig, which, however, remained healthy.

III.—*Mus decumanus* brought to the laboratory dead.

Naked-eye appearances—

Putrid ; organs discoloured ; no enlarged glands.

Microscopically—

Preparations of the spleen and heart-blood showed some bipolar organisms and many putrefactive bacteria.

Animal test—

The spleen was rubbed cutaneously into a guinea-pig weighing 180 grammes. No decrease of weight occurred, but on the tenth day the inguinal glands were slightly enlarged. At the end of a fortnight the animal was chloroformed to death, and the following appearances noted :—The left inguinal gland was somewhat enlarged, but showed no signs of congestion ; the pelvic glands were also larger than normal. There was a very slight local reaction. The spleen was somewhat enlarged, and contained a few grey nodules.

Microscopically—

Preparations from the heart-blood and spleen showed no bacilli.

A culture of the bubo yielded a pure growth of Gærtner's bacillus.

IV.—Liver of a *Mus decumanus* dead of a natural infection of Plague.

The liver of a *mus decumanus*, dead of a natural infection of plague, was covered with small grey nodules, in which fairly numerous plague bacilli were found, on microscopical examination. It was rubbed into

the shaved skin of a guinea-pig, which died in six days of typical plague. Although the animal died of undoubted plague, yet from the heart-blood a pure growth was obtained of numerous colonies, conforming in every way to the type of *B. enteritidis*.

MacConkey gives a similar instance in the case of guinea-pigs dying from experimental inoculations of *B. typhosus* and *B. mallei*.

V.—*Mus decumanus* brought to the laboratory dead.

Naked-eye appearances—

Spleen enlarged and congested; liver pale, and covered with fine necrotic areas; some pleural effusion; lungs hæmorrhagic.

Microscopically—

Spleen—many bipolar bacilli.

Liver—a few bipolar bacilli.

Heart-blood—no bacilli seen.

Animal test—

The spleen and liver were rubbed into a shaved area of skin of a guinea-pig weighing 280 grammes. At the end of a week it was chloroformed to death. Examination showed a small right inguinal gland, with glairy contents, slight cutaneous reaction, and a nodule in the spleen the size of a millet seed. Cultures from heart-blood were sterile, but from the gland and spleen nodule the usual organism was obtained.

From the foregoing details, it may be asserted that organisms of the "Gärtner" type occasionally occur in rats, and that if the cutaneous method in guinea-pigs be employed for purposes of diagnosis, an infection by this organism may result. Confusion from this cause may easily be avoided if the possibility of such an infection be remembered.

Additional emphasis may be given to the fact that the organism may be recovered in pure culture from the heart-blood of a guinea-pig with well-marked lesions produced by another bacillus. Error in diagnosis might, in some cases, possibly result if this circumstance were overlooked.

It only remains, in the concluding part of this paper, to compare the lesions found in diseases caused by other Gärtner-like organisms with those already described. These are, viz.—the bacillus of swine-pest,

B. icteroides, *B. bovis morbificans* Basenau, the bacillus of intestinal diphtheria of Ribbert, *B. psittacosis* Nocard, and *B. diphtheriæ columbarum* Loeffler.

***Bacillus suipestifer*, Kruse. (*Bacillus cholerae suum*, Migula).**

The following description is abstracted from the work of Nocard and L  clainche ⁽⁴⁾.

In the *acute* form of the disease necrotic foci are seen in the sub-mucous tissue of the tongue, and consisting of greyish-yellow caseous masses, which may involve the mucous membrane and finally give rise to ulceration—the ulcers being covered with diphtheritic deposits; the necrosis may extend into the underlying muscles. The tonsils contain h  morrhagic foci or cheesy nodules. The lesions in the stomach and intestine are variable in their intensity, and consist of an exudative inflammation of the mucous membrane, followed by necrosis of the tissues. Usually the spleen is normal; in exceptional cases it contains caseous foci. Multiple nodules of necrosed tissue often occur in the liver.

In the *chronic* form, caseous broncho-pneumonia is met with in addition to the intestinal lesions.

Young animals are especially prone to the disease. The bacillus is pathogenic to the guinea-pig, the rabbit, the white and grey mouse, the white rat and the pigeon.

B. icteroides Sanarelli.—From the researches of Reed and Carrol ⁽⁵⁾, this organism is closely related to *B. suipestifer*, and produces identical lesions in guinea-pigs, mice, rabbits and dogs.

The *B. bovis morbificans* was isolated by Basenau, in 1893, from the muscles and organs of a cow killed on account of puerperal metritis. In several instances the spleen was enlarged, and greyish-white nodules were found in this organ and in the liver. It is pathogenic by feeding, with symptoms of gastro-enteritis to guinea-pigs, mice, rats and calves, and frequently causes, in inoculated animals, numerous miliary purulent and necrotic foci in the liver and spleen.

B. psittacosis Nocard.—Lehmann and Neumann state that it is not clear whether this organism should be placed in the h  morrhagic septic  mia group, though they point out that it has a similarity to

B. enteritidis Gärtner. It is significant that Nocard and Léclainche class it amongst Coli-bacillary infections. Confirmatory evidence of its close relationship to *B. enteritidis* is furnished by MacConkey, who finds that, in their sugar reactions, the two organisms closely correspond.

Nocard states that the lesions in naturally infected animals (parrots) are those of an intense septicæmia. Wolf had previously (1882) described a similar disease in parrots, and had noted in the liver gray or grayish-white hard nodules the size of a millet seed or even larger. These were also present, though smaller and more scattered in the spleen and kidneys. Nocard and Léclainche admit the possibility of the two diseases being identical.

B. der Darmdiphtherie des Kaninchens, Ribbert⁽⁶⁾.—This organism was met with chiefly in pregnant rabbits. Apart from fibrinous peritonitis, the liver and spleen showed numerous minute foci and nodules, in which the liver cells were necrotic. Inoculation reproduced all the morbid changes met with in the spontaneous form of the disease, inclusive of the diphtheritic condition in the intestine.

B. diphtheriæ columbarum, Loeffler. — Lehmann and Neumann investigated a culture obtained from Král, and found that in its morphological and biological characters it exactly resembled *B. enteritidis*. In pigeons affected by the disease, yellow, prominent, fairly adherent spots occur on the mucous membrane of the mouth. These spots consist of a cheesy material. Caseous pseudo-membranes partially cover the mucous membranes of the mouth, pharynx and nasal cavities. The bacillus is pathogenic to the rabbit, when given intravenously. Mice are killed with a septicæmia, whereas in the guinea-pig and dog only an abscess is produced at the site of inoculation.

It is thus evident that organisms belonging to the *B. enteritidis* Gärtner group have a common character in the property they possess of causing necrosis of the tissues selected by them for invasion. Another fact of interest is the wide distribution of these organisms—a fact which is a sufficient justification in attempting to trace their relationship to the type organism of the group, namely, that concerned in a frequent variety of food-poisoning, and indirectly to the still ill-defined class of organisms responsible for those obscure cases of continued fever in man—the paratyphoid bacilli.

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THE RELATIONSHIP BETWEEN THE FACTORS
INDUCING HÆMOLYSIS AND THOSE
INDUCING THE PHAGOCYTOSIS OF RED
BLOOD CORPUSCLES.

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The Relationship between the Factors inducing Hæmolysis and those inducing the Phagocytosis of Red Blood Corpuscles.

A.—HISTORICAL.

Introductory.

THE subject of the phagocytosis of red blood cells is an interesting one from several points of view. It is a phenomenon which occurs in the animal organism under various conditions, and all these processes in which certain cells become the prey as it were of others belonging to the same animal organism must be of much interest to the pathologist.

An interesting example of phagocytosis of red blood cells occurring in the human subject was recently examined by the writer. The case was that of a woman who died of puerperal sepsis six days after delivery. In the large blood sinuses of the uterus could be seen multitudes of mononuclear cells swollen out with ingested red corpuscles, and even amongst the muscle fibres such cells could be observed. Owing to the alteration in form produced by the process of ingestion, it was extremely difficult to say what the exact nature of these cells was. They were large and round, the single nucleus was relatively small, round in shape, and well-stained. Although it is probable that these were mononuclear leucocytes, yet the possibility of their being connective tissue cells had to be considered. In any case it was clear that these cells were acting as scavengers, and were removing the superfluous erythrocytes.

Exactly similar appearances were presented by sections of a carcinoma of the breast in a mouse. These were very kindly brought to the notice of the writer by Dr. F. W. Twort, assistant bacteriologist in

the London Hospital. Many of the alveolar spaces were filled with blood, and lying amongst the red cells could be seen mononuclear leucocytes closely packed with red blood cells.

Much interest, too, is lent to this subject by the close relationship it bears to some other phenomena, which are due to the action of certain body fluids, especially to hæmolysis and agglutination. Of late a considerable amount of discussion has arisen with regard to the relationships of these various phenomena one to another, and in the course of this, much light has been thrown on various questions in connection with the immunity problem.

The histories of hæmolysis and of phagocytosis, especially of red blood cells, are so closely bound together that it may be useful to recount briefly the various stages in the evolution of our knowledge of these phenomena.

The pioneer in this work was Metchnikoff, and although much of his work on the subject has been controverted, yet there can be no doubt that his ideas and observations acted as a great incentive to research on this problem. His work and that of his pupils will repay examination.

Metchnikoff's Views on Macrophages and Microphages.

In the course of his observations on phagocytosis, Metchnikoff was led to the conclusion that various atrophic processes, such as progressive muscular atrophy, senile atrophy, greying of hair, &c., were to be attributed to what he termed the phagocytic action of certain of the body cells. The metamorphosis of various insects and amphibians he considered to be due to the same cause. These processes he attributed almost entirely to the action of *macrophages*, a term which he employed to designate such cells of the fixed tissues as might take on a phagocytic action for animal cells, and the large mononuclear leucocytes. The polymorphonuclear leucocytes he termed microphages, and to these he attributed the power of engulfing micro-organisms.

Metchnikoff's Theory of Cytases and their Origin.

He showed, too, that when one injects blood or other alien cells into the peritoneal cavity or under the skin of animals, phagocytosis of these cells ensues, the phagocytic agents employed being almost exclusively

macrophages. In the peritoneal cavity, some time after such an injection, an accumulation of leucocytes takes place. Amongst these are to be found all kinds of white cells, but the mononuclear macrophages predominate, the polymorphonuclear microphages playing quite a minor rôle. The macrophages take up the alien cells, and proceed to digest them by means of certain ferments which, according to Metchnikoff, they contain. These ferments he termed *cytases*.

Relationship of Cytases to Hæmolysis.

Now, the spleen and lymph glands are organs composed mainly of macrophages, and if one make extracts of such organs with normal saline solution, one obtains, according to Metchnikoff, an extract of macrophages. Such extracts or emulsions cause hæmolysis of red blood cells, *i.e.*, they cause the hæmoglobin to diffuse out. This hæmolytic function of the macrophage extracts, he held, depended on a special substance which they possessed. This substance was destroyed by heat at 56° C. for $\frac{3}{4}$ to 1 hour, *i.e.*, it was thermolabile. Now, since this property of hæmolysis was found to be possessed by the blood serum of animals into which red blood cells had been injected, it appeared to Metchnikoff that the substance in the macrophage extract was identical with that in the serum, and he was of the opinion that in both cases it was an unformed ferment or cytase, which, since it was derived from the macrophages, he termed macrocytase. The cytase which the polymorphonuclear leucocytes contained, he called microcytase.

Views of other Researchers on this Subject.

These views on the cytases and their origin did not long remain unchallenged. It was denied by Korschun and Morgenroth (¹) that the hæmolytic action of macrophage extracts was due to a thermolabile cytase, and in support of this view, they asserted that heating at 56° C did not cause the disappearance of the hæmolytic property of macrophage extracts, and that they even bore the temperature of the boiling point before giving up this power. They showed, too, that the hæmolytic substance in macrophage extracts was soluble in alcohol, and quite different from the true enzymes or cytases, and therefore different from the thermolabile cytase in the blood serum.

Savtchenko and Berdnikoff (²), as well as Donath and Landsteiner (³) and Dömeny (⁴), all controverted the view of Metchnikoff that the macrophages possess a thermolabile enzyme or cytase, which, passing into the blood, affords to this its hæmolytic power. Metchnikoff, however, received support from Levaditi (⁵), who found that such extracts, when fresh, possessed this thermolabile substance, but when old, lost it, and it therefore appeared probable to Metchnikoff that the macrophages contained a thermolabile enzyme which causes dissolution (autolysis) of the cells of such extracts as contain macrophages, and that in the course of autolysis, among the newly formed substances, arises a number of hæmolytic substances of a thermolabile nature.

Levaditi further supported Metchnikoff by showing that fresh extracts of lymph glands contained a macrocytase, and that by means of it one could reactivate sera which had been rendered inactive by heat.

The position held by Metchnikoff was the following. He considered that the macrophages of the lymph glands and other macrophage-forming organs greedily devoured various cellular elements, amongst which were red blood cells. They proceeded to digest these ingested cells, in which process the thermolabile enzymes or cytases possessed by the macrophages played a prominent part. To this thermolabile enzyme or cytase he attributed also the power of causing extra-cellular hæmolysis of red blood cells, and held that it gained access to the blood from the macrophages, and thus lent to the serum its hæmolytic power in cases in which it was possessed of such.

These views were destined to be subjected to considerable discussion and change. Bordet found that in animals into which blood had been repeatedly injected, the hæmolytic action of the macrophage extracts was not increased, while that of the blood serum was much augmented if already present, and produced *de novo*, if not originally there. He demonstrated, too, that instead of a single cytase there were two substances concerned in hæmolysis, one of which, alexine, was present to the same extent in normal as in immune serum. This substance was destroyed at temperatures between 56° C and 60° C. In addition to this complement, however, he showed that in hæmolytic sera there was another substance, which he termed the *substance sensibilisatrice*, from the idea that it made the red blood cells sensitive to the action of the alexine. Bordet showed that it

was much less sensitive to the action of heat and other injurious influences than was the alexine.

The next advance was made by Ehrlich and Morgenroth, who showed that the *substance sensibilisatrice* of Bordet could fix itself on to red blood cells without producing hæmolyis, which was brought about by a thermolabile substance, the alexine of Bordet.

The *substance sensibilisatrice* of Bordet, they termed the *amboceptor*; to the *alexine*, they gave the name *complement*, and showed that the latter did not join on to the red blood cells directly, as Bordet supposed, but probably fixed itself on to the amboceptor, which acted as a connecting link between the red blood cells and the complement, and not as a mordant, making the red blood cell more susceptible to the action of the alexine, which was the view of Bordet.

Revised views of Metchnikoff.

Metchnikoff now accepted the view of the duality of these substances, but proposed, for the sake of clearness, to use the term *fixateur* instead of *amboceptor* or *substance sensibilisatrice*, and the term *cytase* instead of *complement* or *alexine*.

The point, however, which mainly appealed to Metchnikoff was whether the substances producing hæmolysis had any relationship to the phagocytes, and he turned again to the question of the origin of the cytase or alexine, formulating the belief that the macrocytase present in the macrophages of the lymph glands, pancreas aselli and spleen was also to be found in the mononuclear leucocytes of the blood, lymph, and exudates, and that it was identical with the hæmolytic cytase of the blood serum. The grounds for his belief were the following:—

(1) If one immunised a guinea-pig with the blood of a goose, and then injected some goose blood into the peritoneal cavity of the guinea-pig, the alien red corpuscles were quickly hæmolysed without a definite phagocytosis taking place. If one examined the exudate resulting from such a procedure, one found but few leucocytes, and even those were in a state of inactivity, being non-mobile, clumped together, and unable to engulf foreign bodies. They were, in fact, undergoing a process of dissolution, which Metchnikoff termed phagolysis. According to Metchnikoff, it was possible to prevent the occurrence of phagolysis by

injecting sterile broth or normal saline solution into the peritoneal cavity 24 hours previous to the injection of red blood cells. One thus inured the phagocytes to insult, and no phagolysis was produced on the subsequent injection of red blood cells. As a consequence, when the erythrocytes were injected, phagocytosis went on readily without the occurrence of extracellular hæmolysis. The red cells were hæmolysed only in the interior of the phagocytes.

From these observations Metchnikoff concluded that the hæmolytic substances in the peritoneal exudate were set free from the leucocytes through the occurrence of phagolysis, and that, as soon as the latter was prevented, hæmolysis ceased to occur in the peritoneal fluid and took place in the interior of the leucocyte.

(2) Metchnikoff further asserted that the presence of alexine or complement or cytase, which occurs in blood drawn from an animal into an ordinary glass vessel, was brought about by injury to mononuclear leucocytes, many of which were broken down during the process of clotting, and thus set free their cytases. This opinion was supported by the experiments of some of his pupils ⁽⁶⁾ who found that if a vein were ligatured and then excised and suspended, the plasma, which could be pipetted off, contained no cytase; and that, further, if blood were drawn into *paraffined* glass tubes, no cytase was present, since in neither case had any destruction of macrophages occurred. These views, which were subsequently supported by the experiments of Tarassewitch ⁽⁷⁾ and Levaditi, have recently been controverted by Lambotte and Stiennon ⁽⁸⁾, who have maintained that even when the methods recommended by Metchnikoff were carefully carried out by them, the results obtained by him and his pupils did not ensue. As a result of their experiments, they denied the intra-cellular nature of the cytases. The question of the origin of the cytases is a difficult one, and cannot as yet be regarded as definitely settled.

The Relationship of the Substances in the Serum producing Hæmolysis to the Phenomenon of the Phagocytosis of red blood cells.

After this short resumé of the history of the substances in serum which produce hæmolysis, it will be proper to discuss the relationship of

these substances to the phenomenon of the phagocytosis of red blood cells. Our knowledge of this subject was considerably enlarged by Savtchenko (⁹), who immunised rabbits against the red cells of the guinea-pig. The serum of the former naturally became hæmolytic for the red blood cells of the latter. Savtchenko showed that if one injected sterile bouillon into the peritoneal cavity of a guinea-pig, and 24 hours afterwards the washed red cells of another guinea-pig, along with a quantity of the heated serum of the immune rabbit, no hæmolysis took place, but phagocytosis of these red cells was marked. On the other hand, in a control guinea-pig injected with the red blood cells, but without the immune serum, no phagocytosis took place. When he injected a fresh non-immune guinea-pig with the red blood cells of another guinea-pig along with the heated immune serum, but without having prevented phagolysis by the appropriate measures, hæmolysis took place owing to the cytase or complement being set free by the destruction of the macrophages, consequent on the injection. On injecting a larger quantity of the red cells, both hæmolysis and phagocytosis occurred.

He also performed experiments *in vitro*, using the same factors as before, and found that under the influence of the immune hæmolytic serum, deprived of its alexine by heat, the red blood cells were engulfed by the leucocytes of the guinea-pig which he used as the phagocytic agents, and that no extra-cellular hæmolysis occurred.

From his experiments, Savtchenko concluded that in the serum of a rabbit rendered immune towards guinea-pig's blood, some body was present which made the erythrocytes an easy prey to the leucocytes of another guinea-pig. This was not the alexine, since the property was possessed by sera which had been deprived of that body by heat, and therefore Savtchenko concluded that it was the specific hæmolytic *fixateur*, the *amboceptor*, which induced the phagocytosis of red cells. This body, he asserted, could act in two ways. It could either attach itself to the phagocyte and stimulate it to engulf the red blood cells, or, by attaching itself to the erythrocytes, could render them an easy prey to the phagocytes. Metchnikoff agreed with Savtchenko, Taraskevitch and Levaditi, that the specific fixateur or amboceptor induces phagocytosis.

Relation of Fixateur to Opsonin.

This subject acquired a fresh interest from its bearing on the work of Wright and Douglas (¹⁰), Bulloch and Atkin (¹¹) and others, on phagocytosis.

Wright and Douglas described the power which the blood serum has of inducing the phagocytosis of micro-organisms as the "opsonic" property, and the substance possessing this as the "opsonin," which, in their opinion, was a substance not previously described, and which acted on the micro-organisms or other bodies to be phagocytosed, and not on the phagocytes.

It was subsequently asserted by Dean (¹²) and others that this was not a new substance, but that it was identical with the fixateur, using that term in the sense in which it was employed by Savtchenko, *i.e.*, regarding it as identical with the amboceptor.

Barratt (¹³), however, has since shown that the specific hæmolytic fixateur may be absent in an immune serum, and that, nevertheless, a substance inducing the phagocytosis of red blood cells of the kind employed for immunisation may be present. This substance he classed with the "opsonins."

The point at issue, then, is whether the substance which induces the phagocytosis of red blood cells is identical with the specific hæmolytic fixateur or amboceptor, and the following experiments were carried out with a view to elucidate this question.

B.—EXPERIMENTAL.

If the process of phagocytosis of red blood cells be due to the action of the hæmolytic amboceptor, as suggested by Savtchenko, then there are two ways in which it may act.

(1) It might act alone by attaching itself to the red blood cells, thus rendering them liable to be phagocytosed. This theory is supported by Savtchenko, Metchnikoff, Dean, &c.

(2) It might, however, as Dean has also suggested, be aided in this action by a complement, but if the complement were destroyed, might still be capable in itself of inducing phagocytosis.

In dealing with the first point, a question naturally suggesting itself was how do these two substances, viz., that which induces phagocytosis and the hæmolytic amboceptor behave towards heat? If the one be partially destroyed while the other is not, then naturally one would conclude that the two are not identical.

In order to procure information on this point, the following mode of procedure was adopted :—A rabbit was immunised against the red blood cells of the ox. The serum of this rabbit became hæmolytic for these red blood cells, '002 c.c. ultimately producing, when fully complemented with fresh guinea-pig serum, complete hæmolysis of 2 c.c. of a 5 % suspension of the red cells, after two hours at 37° C. and 12 hours at room temperature; '0001 c.c. produced a trace of hæmolysis under the same conditions.

The following series of tests were directed towards ascertaining whether the hæmolytic amboceptor (fixateur) was thermolabile or thermostable.

Two separate portions of the serum were taken, one being heated at 55° C for 15 minutes, the other being left unheated. Two series of corresponding dilutions were made from these, and to each tube 2 c.c. of a 5 % suspension of the washed red cells of the ox were added with '2 c.c. of fresh guinea-pig serum as complement. Both series were kept at 37° C. for two hours, and subsequently at 0° C. for 24 hours. The corresponding dilutions showed exactly corresponding degrees of hæmolysis, even when examined by means of von Fleischl's hæmometer. These results, which are given in detail elsewhere (¹⁴), and which were confirmed by repeated subsequent experiments, indicate that there is no destruction of the hæmolytic amboceptor after an exposure of 15 minutes to a temperature of 55° C.

Similar experiments were also made on the substance inducing phagocytosis of red blood cells.

These effects were very different from those obtained by heating the hæmolytic amboceptor.

The method adopted for the performance of the phagocytic tests was that invented by Wright for micro-organisms. Equal parts of serum, of the usual 5 % suspension of red blood cells and of the washed human leucocytes were mixed in capillary pipettes, and placed at 37° C. for 15 minutes, films being then made, and stained with Leishman's stain.

In such tests it is a matter of some difficulty to compare heated and unheated sera when undiluted, since the hæmolysis produced by the latter is apt to obscure the phagocytosis, but by means of a method of dilution which was adopted, it was found possible to abolish the action of the complement, and thus suppress hæmolysis, while phagocytosis could still be observed. By the employment of such a method of procedure it was possible to demonstrate that in corresponding dilutions unheated serum gave a higher phagocytic count than did heated.

The following experiment illustrates this:—The unheated immune rabbit's serum was diluted so as to give proportions of 1 in 15, 1 in 20, 1 in 30 and 1 in 60, and of each of these dilutions, one part was mixed in a capillary pipette with one part of a 5 % suspension of the washed corpuscles of an ox and the part of washed human leucocytes, the final dilutions thus resulting being 1 in 45, 1 in 60, 1 in 90 and 1 in 180 approximately. At the same time a similar series was made with serum which had been heated at 55° C. for 15 minutes. A control was made, consisting of 1 part of .85 % saline solution, one part of the suspension of red cells and one part of washed human leucocytes. Each mixture, as soon as it was made, was placed for 15 minutes at 37° C., films being then made, and stained in the usual manner. In each case, 100 polymorphonuclear leucocytes were counted, and the degree of phagocytosis estimated from the percentage of the leucocytes containing red blood cells. This method was found to be more accurate in the case of red blood cells than that of estimating the average number of the red corpuscles taken up per leucocyte, as it was in many instances difficult to distinguish the individual erythrocytes.

In this particular experiment, the results were as follows (the percentages refer to the leucocytes containing red blood cells):—

Dilution.	Unheated serum.	Heated serum.
1 in 45	65 per cent.	20 per cent.
1 in 60	60 "	6 "
1 in 90	21 "	4 "
1 in 180	8 "	—

Saline control, 5 per cent.

From these and similar results, it is to be assumed that the substance inducing the phagocytosis of red blood cells is partially destroyed by heat, and that, therefore, it cannot be identical with the hæmolytic amboceptor or fixateur.

This is corroborated by the results of experiments with normal hæmolytic sera. The serum of a normal guinea-pig is slightly hæmolytic for the red blood cells of the rabbit. The serum of a normal guinea-pig, the washed red blood cells of a rabbit, and washed human leucocytes, were employed for phagocytic tests *in vitro*. It was found that many of the leucocytes employed as the phagocytic agents contained blood shadows, that is, instead of red blood cells they contained clear circular spaces. In most films, if 100 polymorphonuclears were counted, a few contained one or two apparently unchanged red cells, and a few contained erythrocytes, more or less hæmolyzed. It was thus possible to trace the gradations between apparently normal red cells lying in the polymorphonuclear leucocytes and clear round spaces representing the erythrocytes in a hæmolyzed condition.

If, however, with such a serum heated at 50° C. - 60° C. for 15 minutes, phagocytic tests were performed, no evidence of phagocytosis was observed. Neither blood shadows nor red blood cells were to be seen in the leucocytes, except in a very few instances, where one or two polymorphonuclears contained a single unchanged erythrocyte. The opsonic action of the serum had been abolished. Yet there was undoubtedly a considerable amount of hæmolytic amboceptor present, since it is not destroyed by heat at the temperature mentioned above, and therefore we cannot assume that abolition of the opsonic effect of the blood serum is due to destruction of the hæmolytic amboceptor.

That a destruction of the "opsonin" takes place may be seen from the following experiment:—The opsonic power of the serum of a guinea-pig unheated on the one hand and on the other heated at 55° C. for 15 minutes, on the washed erythrocytes of the rabbit, was determined in the usual manner twice a day for three consecutive days, the amount of phagocytosis being estimated by the percentage of polymorphonuclears containing blood shadows or red blood cells more or less hæmolyzed.

Estimation.	Unheated serum.	Heated serum.
1.	37 per cent.	0 per cent.
2.	47 "	0 "
3.	40 "	0 "
4.	47 "	0 "
5.	41 "	0 "
6.	48 "	0 "

It was evident from these figures as well as from many similar results that a great destruction of the normal "opsonin" took place at 55° C.

Again the unheated blood serum of the eel is strongly hæmolytic for most red blood cells, yet, when heated, it appears to exert no opsonic effect on red blood cells for which it is hæmolytic. It is impossible to perform phagocytic tests with eel's serum in the unheated condition, since it is markedly toxic for leucocytes, and, by dissolving these, renders such tests futile.

Example.—An eel's serum, of which when unheated .05 cc. produced almost complete hæmolysis of 2 cc., of a 10 % suspension of guinea-pig corpuscles, after 2½ hours at 37° C., was used. Such a serum when heated no longer produced hæmolysis, nor had it any power of producing phagocytosis of the red blood cells of the guinea-pig when tested in the usual way. In films in which 100 polymorphonuclears were enumerated none contained any red cells, although we may assume that hæmolytic amboceptor was present in a very large amount.

In diluted immune sera the hæmolytic amboceptor may be present, but without being able to render the corresponding red cells suitable for phagocytosis.

Experiment.—Into two series of suitable test tubes .01, .005, .003, .002 cc. of the immune rabbit's serum was placed. To each tube was added 2 cc. of a 5 % suspension of the washed red cells of the ox, and all were made up to equal volumes with .85 saline solution. One series was complemented with fresh normal guinea-pig serum, the other being left uncomplemented. The immune serum was present in the mixtures in the proportions of 1 in 220, 1 in 450, 1 in 730, and 1 in 1100 respectively. A control consisted of a similar mixture containing the serum in the proportion of 1 in 10. All the mixtures were placed for 2 hours at 37° C. At the end of that time the following results were noted :—

Amount of serum.	Complemented series.	Uncomplemented series.
.01 cc.	Hæmolysis almost complete.	No hæmolysis.
.005 cc.	" marked.	"
.003 cc.	" slight.	"
.002 cc.	" slight.	"

The tubes of the uncomplemented series and the control were then well shaken up and centrifugalised. The supernatant fluid was pipetted off, and replaced by normal saline solution. This was repeated thrice.

The deposits, thus freed from all traces of serum, were then mixed with equal parts of '85 % saline solution, and were rapidly drawn up and down in capillary pipettes to produce a uniform suspension.

Equal quantities of each deposit and of washed human leucocytes were mixed in capillary pipettes, which were then kept at 37° C. for 15 minutes. Films were made and stained in the usual manner.

In the control the phagocytosis of the red cells was marked, 93 % of the leucocytes containing red cells, but in the dilutions of 1 in 220, etc., no phagocytosis could be seen, although 100 leucocytes in each case were counted.

It was scarcely to be supposed that the absence of phagocytosis was to be attributed to lack of the hæmolytic amboceptor in these dilutions, since hæmolysis took place, in one case to a marked extent, in the corresponding complemented tubes under conditions similar to those in which the uncomplemented series used in the phagocytic tests was placed.

The natural conclusion to be drawn from these experiments is that the hæmolytic amboceptor is not identical with the substance inducing the phagocytosis of red blood cells.

As was indicated previously in this paper, the possibility has been suggested by Dean that the opsonic action may be induced by a complement acting through an amboceptor.

The complementary part of the action would be suppressed by heat, while the amboceptor might go on acting in such a way as to still cause phagocytosis.

It is very difficult to disprove this theory by direct experiment for several reasons. The complement is destroyed at the same temperature as the opsonin. If one add fresh complement, *i.e.*, fresh guinea-pig serum, it is impossible to exclude the effect of the inherent opsonic property it possesses. Thus it is impossible to say how far the increase in opsonic power in such a case might be due to a complementary action, apart from the opsonic property possessed by fresh sera generally.

To this view of Dean's, however, there are various objections.

In the first place, we have as yet no example of such an action of immune substances. In the case of hæmolysis, which is a typical example of the action of amboceptor and complement, when we destroy the complement by heat, no hæmolysis takes place, notwithstanding the

presence of a large amount of amboceptor. On the other hand, in highly immune sera, the opsonic property of the serum, after heating, may still be present to a marked extent, and in general, as is shown later on, the more highly immune a serum is the less is the difference in opsonic effect between the heated and unheated sera. Again, from the dilution experiments recorded above, it is evident that, after the serum has been diluted to such a degree that the complement is no longer present in an amount sufficient to cause hæmolysis, yet the serum in the unheated state exercises a greater opsonic effect than in the heated condition in corresponding dilutions.

Even if it be admitted that Dean's suggestion is feasible, yet to explain the action one would have to suppose that the amboceptor possesses two groups, one of which is a complementophilic, the other a special function group. Thus, though the action of the complement were suppressed, such an immune substance, in virtue of the action of its special function group, might still be capable of inducing phagocytosis. Even admitting this, the fact would still remain that the substance is a new one, and not of the exact nature of any immune substance previously described, and to such a substance the term opsonin might reasonably be applied.

There are two further points to which reference may be made. The first is the difficult question of the leucocytic origin of the complement. Time has not permitted of experiments on this problem, but one observation may be referred to. If one perform phagocytic tests in the usual way, with an unheated hæmolytic serum, whether normal or immune, human leucocytes, and the red blood corpuscles of an ox, guinea-pig, etc., blood shadows appear in the leucocytes. This phenomenon appears almost instantaneously at 37°C . In the case of unheated guinea-pigs' serum and rabbits' corpuscles, it was found that, after three minutes at 37°C ., blood shadows appeared in the leucocytes. On the other hand, using an immune heated hæmolytic serum after one hour at 37°C ., the red corpuscles in the leucocytes were apparently still unchanged. No hæmolysis had taken place. But if, as Metchnikoff and his followers have stated, the phagocytes contain the cytases or alexines, then one should have expected hæmolysis to take place inside the leucocytes. The above observations, however, tend to show that such is not the case.

The last point to which reference will be made is the question of the destruction of the opsonin by heat. Wright and Douglas (*loc cit.*), as well as Bulloch and Atkin (*loc cit.*) and Hektoen and Ruediger⁽¹⁵⁾, working with normal sera with Wright's method, found that this substance was almost entirely destroyed by heat at 50° C. - 60° C. Dean repeated this work not only with normal, but also with immune serum, using, however, a somewhat different mode of procedure, and found that in normal, but especially in immune sera, there was less destruction than the authors mentioned had supposed.

The following experiment illustrates this point, and shows that the more highly immune a serum becomes, the less is the apparent destruction of opsonin. This experiment was only a trial one, directed to find the relationship of the curves of hæmolysis and phagocytosis to one another. Owing to the fact that the author had perforce to abandon this work, no further experiments on the point could be carried out, but from the results it is evident that the higher the immunity becomes the less was the apparent destruction of the opsonin. The opsonic action is limited in two ways. On the one hand, a certain number of leucocytes constantly seem to be incapable of exercising, or exercise only with difficulty, the property of phagocytosis, and on the other the capacity of the leucocytes is not unlimited, especially for bodies of the size of red blood cells. Thus there are two limitations. Suppose, *e.g.*, that in a given case, 90% of the leucocytes used for a test are capable of acting as phagocytes. Now, a highly immune serum, even in the heated condition may be so active, and make the erythrocytes so easy to engulf, that 90% of the leucocytes may contain red blood cells. But although the unheated serum may be much more powerful than the heated, yet it cannot produce more phagocytes, since the limit (90%) has been reached by the heated serum. The greater the opsonic content of an immune serum is, the more will this limitation come into play.

The method of experimentation was as follows:—A normal guinea-pig was taken. Of its serum, one part was mixed in capillary pipettes with one part of a 5% suspension of the washed red blood cells of the rabbit and one part of washed human leucocytes. Phagocytic tests were then performed in the usual way. The percentage of leucocytes containing blood shadows was counted in the case of heated as well as unheated serum. At the same time serum of a control normal

guinea-pig was employed in the same way. Hæmolytic tests were performed by means of Wright's tubes, and without additional complement.

After testing the serum of the guinea-pig, 3 cc. of the washed corpuscles of a rabbit were injected intraperitoneally, and 5 days afterwards 5 cc. of the same material. The serum was examined, as a rule, daily for three weeks, from the time of the first injection, and the results are to be seen in the accompanying chart.

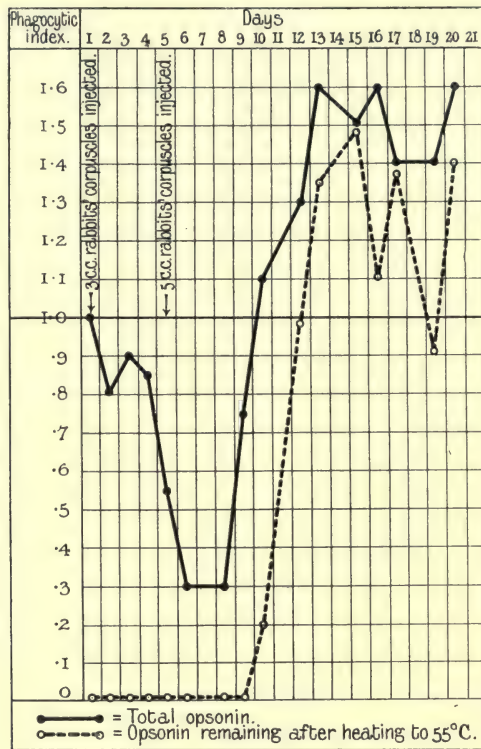


Fig. 1.

The method of estimation was as follows :—

The opsonic effect of the serum of the immunised guinea-pig when unheated, and when heated at 55° C. for 15 minutes, was compared with that of the control animal.

In each case equal parts of the serum, of a 5 % suspension of the washed corpuscles of a rabbit and of washed human leucocytes, were employed.

In the case of the unheated sera the number of leucocytes in 100 containing blood shadows was taken as a criterion, and in the case of the heated serum the number of leucocytes containing red blood cells, since, in the latter, no shadows appear.

The numbers so found for the heated and unheated sera of the immune animal were divided by the number found for the control, and thus figures were obtained representing the opsonic index. Thus, if in the case of the unheated control serum 50 % of the leucocytes contained blood shadows or red blood cells, while with the immune serum in the unheated condition 80 %, and in the heated condition 60 % of the leucocytes showed erythrocytes or shadows or both in their interiors, then the opsonic index, in the case of the heated serum, was 1·2, and in that of the unheated 1·6.

Testing in this way, a curve (Fig. 1) was obtained from which one deduces that the more highly immune the serum became the less was the apparent destruction of the opsonin by heat.

This experiment was only a trial one, but the principle involved seems to be worthy of further exploitation.

I have to express my warmest thanks to Dr. William Bulloch of the London Hospital for very much kind advice and assistance throughout my work on this subject.

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AN EXPERIMENTAL ENQUIRY INTO THE
RELATIONSHIP OF LEUCOCYTOSIS TO
THE OPSONIC CONTENT OF THE BLOOD
SERUM.

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An Experimental Enquiry into the Relationship of Leucocytosis to the Opsonic content of the Blood Serum.

I.—Introduction.

THE following experiments were undertaken with a view to determine whether and to what extent the opsonic content of the blood serum, towards various micro-organisms, is influenced by changes in the number and character of the circulating leucocytes. The general association of leucocytosis with natural and experimental bacterial infection, has long been observed as a more or less constant phenomenon, without any very exact conclusions having been reached regarding the precise rôle played by such leucocytic variations.

It is obvious that this response on the part of the white cells to infection of the organism, must form a very important link in the chain of protective mechanisms called into play by the animal body, but, where opinions have differed and still differ, is in the interpretation of the comparative rôles played by the cellular elements and the surrounding fluid medium, and in the rationale of their inter-dependence, if such exists.

It has been shown by Wright and Douglas (¹) and Bulloch and Atkin (²) that in comparing *in vitro* the opsonic content towards micro-organisms, of normal with pathological sera, the variant is the serum, while the leucocyte may be regarded as a more or less indifferent factor: that is to say, normal leucocytes may be employed instead of those corresponding to the serum to be tested, without incurring any appreciable error.

It may here be observed, however, that in certain diseases of the hæmopoietic system, one of us (Ledingham) has put on record evidence

showing that the ultimate agents concerned in phagocytosis—the neutrophile leucocytes—may vary greatly in their phagocytic power, quite apart from changes in the opsonic content of the serum. But these results, though interesting, do not affect the main proposition that, under conditions which do not primarily involve the hæmopoietic system, the serum is the main variant.

If then the phagocytic power of the neutrophile leucocyte remains approximately constant both in normal and pathological conditions, two questions arise which we shall endeavour to answer in this paper.

1. Will the presence of a leucocytosis in the circulating blood have any effect on the opsonic content of the serum, either in the way of raising or lowering it? And, in this connection, is there any evidence pointing to a probable origin of opsonic substances from products of degenerated leucocytes?
2. If the leucocyte, normally, has a constant phagocytic property, does this relationship hold good in the stage of hyperleucocytosis?

Both of these questions are obviously of great interest from the point of view of vaccine-therapy the immediate effect of which, at least, is to cause some disturbance of the blood picture. For the production of experimental leucocytosis, we decided that it was of great importance to choose fairly simple chemical bodies such as sodium cinnamate, tallianine, etc., and to avoid bodies of more complex chemical constitution such as bacterial proteins and sterile culture emulsions, which, though causing on inoculation definite leucocytic changes (Römer⁽³⁾, Schlesinger⁽⁴⁾, Holmes⁽⁵⁾, and others), have been shown by Wright and Douglas to raise the opsonic content of the serum.

This method of attacking the problem by inoculating leucocytosis-producing substances has been much employed and elaborated since Buchner's⁽⁶⁾ first application of it to the problem of the nature and origin of the alexine of normal serum.

The work of Nuttall⁽⁷⁾, Nissen⁽⁸⁾ and Buchner, which went to show the presence of a thermolabile bactericidal body in cell-free serum, was followed by numerous researches, notably of Hankin⁽⁹⁾, Denys⁽¹⁰⁾, Wasserman⁽¹¹⁾, Buchner (*loc. cit.*), Schuster⁽¹²⁾, Schattenfroh⁽¹³⁾, etc., which had for their object the demonstration of bactericidal substances elaborated by the leucocytes themselves.

Hankin and Kanthack (*loc. cit.*), Denys and Kaisin (¹⁴), showed that the bactericidal power rose or fell according to the variations in the number of leucocytes whether intravascularly or extravascularly or in exudates; but from these experiments, involving as they did the continued vitality of the leucocytes, and thus of continued phagocytosis, no definite conclusions could be drawn as to the rôle played by each in the process.

Buchner and Schuster employed substances like aleurone emulsions or gluten-casein, which they injected into the pleural cavity, thereby obtaining large quantities of fluid rich in leucocytes. By a process of alternate freezing and thawing the leucocytes were killed, and the mixture was found to have stronger bactericidal powers than the blood serum.

Other methods of obtaining extracts of dead leucocytes were employed by Löwit (¹⁵) and Schattenfroh, the latter of whom was led to the conclusion that the bactericidal substance obtained from dead leucocytes is relatively thermostable, and is not identical with the blood alexines.

The whole question of the relationship of the bactericidal body in leucocytic extracts to the alexine is still *sub judice*, and, further, the view of Metchnikoff, that alexine is formed only when the leucocytes are disintegrated intravascularly or *in vitro* in the process of coagulation, has been disputed strongly by various authors, and more recently by Lambotte and Stiennon (¹⁶). These latter observers have recently employed very careful methods for the preparation of plasma entirely free from leucocytes, which, however, are not destroyed in the process. Their results go to show that alexine circulates as such free in the plasma, for they find that the bactericidal power of the plasma so obtained is equal to that of the corresponding serum after coagulation.

The above considerations have an interest for us in this connection, only in so far as the opsonic substance has been regarded by Wright and Douglas and Bulloch and Atkin (*loc. cit.*) as a simple thermolabile substance like the alexine, but which, in marked contradistinction to the latter, does not require for its efficient action the co-operation of a thermostable immune body.

The latter proposition has, however, been recently disputed by Dean (¹⁷), who regards the opsonic substance or the substance concerned in the preparation of bacteria for phagocytosis as essentially thermostable,

and which, though capable of acting to a certain extent independently, may have its action enormously reinforced by co-operation with a thermolabile complement. In a recent paper by R. D. Keith (¹⁸), the view is also put forward that the immune opsonin is partially thermostable, but it is not identical with the hæmolytic amboceptor, as Dean believed. To explain the residual opsonic action that takes place when immune serum has been heated, Keith suggests that, in addition to its cytophilic and complementophilic groups, the hypothetical opsonic amboceptor may possess a special thermostable opsoniphoric group, enabling a certain amount of action to proceed in the absence of complement. Into the problems involved in this highly theoretical subject, it would be out of place to enter here.

We take as our main premiss, the standpoint, that the opsonin is a simple thermolabile body residing in the blood serum, whose action *in vitro* is exerted solely on the micro-organisms concerned, and is entirely independent of the leucocyte.

The ultimate origin of the opsonic substance, like that of the bactericidal and other protective bodies, is difficult to determine with precision, and it was felt that light might be thrown on the subject, not so much by Buchner's method of analysing separately the cellular and fluid portions of the plasma as to their opsonic content (though this also was attempted), as by estimating what influence, if any, was exerted by the presence of hyperleucocytosis on the opsonic content of the plasma, or, more correctly, of the cell-free serum after coagulation.

If the opsonic substance is ultimately derived from leucocytic degenerative products, one would expect it to be profoundly influenced by conditions in which the white cells are in great excess in the blood stream.

The injection of many substances differing widely in chemical constitution has long been known to be followed by a period of hyperleucocytosis, during which the natural resistance to infection is increased. Indeed, it is probable that in many of the so-called cases of cross-immunisation or vaccination with sterile emulsions of bacteria, whose origin is entirely different from those against which they exert a protective action, the results obtained are attributable solely to the leucocytic variations produced by them.

It will be convenient here to summarise briefly the protective effects

that various authors have recorded by the employment of substances producing leucocytosis.

The first experiments in this direction were made by Loewy and Richter (¹⁹), who succeeded in protecting rabbits against experimental pneumococcic infections, by injecting substances like spermin, pilocarpin and albumoses. These experiments were further extended by Jacob (²⁰), who also noted that animals inoculated in the leucopenic period, following the injection of albumoses, always succumbed, while those infected during the period of hyperleucocytosis were markedly protected.

Hahn (²¹) employed yeast-nuclein in dogs and tuberculin in man, and showed that the blood drawn during the stage of hyperleucocytosis had a higher bactericidal power than normal blood. The period of immunity was, however, always very short, and in striking contrast to that obtained by previous inoculation of extracts or sterilised cultures of the organism employed for infection.

Very complete and convincing experiments were made by Pfeiffer and Issaëff (²²) in experimental cholera infection. They showed that the inoculation of guinea-pigs with nuclein, serum of cholera convalescents, serum of healthy persons, bouillon, urine, and salt solution, gave a protecting power which diminished in the order of the substances named.

Other substances as tissue-fibrinogen (Wooldridge (²³)), testicle emulsion (Zacharoff (²⁴)), thymus and lymph-gland extracts (Brieger, Kitasato and Wassermann (²⁵)), gluten-casein, legumin (Buchner, *loc. cit.*), were found, on injection, to give varying degrees of protecting power, attributable to the hyperleucocytosis produced by them.

With these and many other substances, different observers have, however, frequently obtained different results, a fact which is not surprising when one considers the well known differences in the individual response to such substances, the mode of administration, and the amount inoculated.

In all these instances of increased natural resistance associated with hyperleucocytosis, it would be exceedingly interesting to ascertain with exactitude how the two bodies amboceptor and complement—concerned in the destruction of micro-organisms—were respectively influenced by the leucocytic variation. Very few results, however, are forthcoming in this direction, but it appears from the work of Nolf (²⁶), Müller (²⁷), Wassermann (*loc. cit.*) and Longcope (²⁸) that it is the complement and

not the amboceptor which is most markedly influenced by the injection of leucocytosis-producing substances.

Bulloch has also shown that the inoculation of sodium cinnamate raises the complement content, but leaves the hæmolytic amboceptor quite uninfluenced.

We shall refer later to the bearing of these facts on the experimental results obtained by us, and which are presently to be detailed.

Technique of Experiments.

The effect of all substances employed in the production of leucocytosis, was tested both on rabbits and guinea-pigs, the inoculation being made intraperitoneally in the case of the latter, and either intravenously or intraperitoneally in the case of the former. In the former, also, the subcutaneous method was once or twice employed. Immediately before the inoculation, samples of blood for counting purposes (total and differential leucocyte counts), and estimation of opsonic content of serum, were drawn from the posterior auricular vein. In the guinea-pig one occasionally has a little difficulty in obtaining satisfactory amounts of blood from the ear, but, with some practice, one can usually obtain quite a large supply by making a smart puncture in the vein situated close to the margin of the ear, and exercising a little pressure. At the same time blood was also drawn from another animal, to act as control in the estimation of the opsonic index in the experimental animal. Whenever a sample of blood was drawn from the experimental animal during the period of observation, a similar sample was drawn from the control animal. In many cases this was found to be unnecessary, as we shall show (*cf. infra*).

The blood counts were made by the usual hæmatological methods. For the opsonic determinations the technique recommended by Wright was carried out as follows :—

1. The blood sample was collected in a glass capsule with a recurved limb. When coagulation had set in, the tube was hung in a centrifuge, which brought about the separation of the clear serum.
2. In most cases the leucocytes employed were of human origin, and were obtained by allowing the blood to run into a glass capsule

containing sodium citrate (1 % dissolved in .85 % NaCl solution). The citrated blood was then centrifugalised, the supernatant citrated plasma being removed. The deposit of corpuscles was subsequently washed in a large volume of saline solution, and finally, the corpuscles were brought down as a deposit in the centrifuge, the supernatant saline solution being then removed.

3. A uniform emulsion of the microbe to be tested was made in saline solution. The serum, corpuscles and microbe emulsions were then introduced in definite volumes into capillary pipettes, and, after thorough mixing of the contents, the latter were sealed, placed in the incubator for 15' at 37° C, at the end of which time films were made of the mixtures, and after suitable staining (Leishmann, or Ziehl-Neelsen), the number of bacteria ingested by a number of leucocytes was counted. In comparison with the number ingested in the presence of a normal serum this yielded the phagocytic index :

$$\frac{\text{Number of bacteria ingested by leucocytes in the presence of given serum}}{\text{Number of bacteria ingested by leucocytes in the presence of normal serum}} = \frac{\text{opsonic}}{\text{index.}}$$

It is manifestly impossible for one person satisfactorily to carry out experiments of the kind detailed in this paper. We accordingly arranged it so that one of us (L) performed the whole of the blood counts, the other (B) devoting himself to the determinations of the opsonic content. Each worked separately, and at the end of the experiment the results were compared.

II.—Description of the normal blood-picture in the Rabbit and Guinea-pig and its physiological variations.

RABBIT.

The total leucocyte count varies greatly in different rabbits. Goldscheider and Jacob⁽²⁹⁾ found a variation of 8000 to 14,000 leucocytes per cub. mm., while Schlesinger (*loc. cit.*), from a series of 100 rabbits, obtained an average count of 10,800, with a variation of 5000 to 17,000. Schulz's⁽³⁰⁾ average was 9900. Rieder⁽³¹⁾, Löwit⁽³²⁾, Ruzicka⁽³³⁾,

obtained averages of 8844.9, 15,998.6, and 14,677.7 respectively. Brinckerhoff and Tyzzer (³⁴) found a variation of 4000 to 12,000.

In our own experiments we obtained from a series of 25 normal healthy rabbits an average leucocyte count of 10,533 with a variation of 4500 to 17,200.

Far more important than these large variations in different rabbits, is the consideration of those minor variations which may occur in the counts of individual animals independently of experiment, and which, if neglected during experiments on leucocytosis, may give rise to fallacies.

It would appear sufficient to ascertain the leucocyte count of the animal on three or four days prior to the experiment, such counts being made at the same hour every day, and under similar conditions as to feeding or fasting.

Brinckerhoff and Tyzzer (*loc. cit.*) recommend more elaborate precautions. Before experiments on leucocytosis, they make careful observations of the influence of fasting or feeding on the leucocyte count, and finally select those rabbits only whose leucocytes decrease during a period of fasting. For a short experiment, the animal should be allowed to fast for 12 to 20 hours, and if during this time the leucocyte count does not rise, the experiment may be carried out with the assurance that for a time the variations in the leucocyte count will be unworthy of consideration. Other influences to be reckoned with, such as loss of body heat, shock and pregnancy, are obvious. In our experiments it did not appear to us either advisable or humane to keep the animals without food even during short experiments lasting six to eight hours. After the inoculations the animals invariably showed a loss of appetite throughout the course of the experiment, so that any small errors due to digestion-leucocytosis might be neglected. Further, as we were able to demonstrate in many control animals, the slight rises, due to feeding, came well within the experimental error with our available counting methods.

Pohl (³⁵), who investigated this question of digestion-leucocytosis, came to the conclusion that the phenomenon was marked only in those animals such as the dog, which received meals once or twice a day, and was quite inconsiderable in animals like the rabbit, whose stomachs were continually full.

Regarding the leucocyte types met with in rabbit's blood, the classification usually adopted is as follows:—

- (1) Lymphocytes.
- (2) Large Mononuclears.
- (3) Amphophiles or Polymorphonuclears. These correspond in general morphology and function to the polynuclear neutrophils of man, but their granules are smaller and more ovoid, and, unlike those of man, give a distinctly oxyphile reaction. For the sake of convenience and uniformity of terminology, we shall, in the course of this paper, refer to these cells as neutrophils, and apply the term neutrophilia to the condition in which these cells are in excess.
- (4) Eosinophiles with large ovoid oxyphile granules.
- (5) Mast cells.

As to the relative numbers of these cells in the circulating blood, Löwit (*loc. cit.*) found 79·7 % polynuclears and 20·3 % mononuclears, while Hirschfeld (³⁶) found the lymphocyte percentage equal to or even greater than the polynuclear percentage.

Schlesinger (*loc. cit.*) obtained an average polynuclear percentage of 49, with a variation of 40 to 60.

Brinckerhoff and Tyzzer (*loc. cit.*) give more complete numerical relations, which will be seen to correspond closely with our own results obtained from a series of 25 rabbits—

	Brinckerhoff and Tyzzer.	Ledingham.
Lymphocytes	45·55 per cent.	44·8 per cent.
Large Mononuclears	2·8 "	6 "
Amphophiles	40·50 "	42·4 "
Eosinophiles	0·5-1 "	1 "
Mast cells	4·8 "	5·8 "

Does any marked variation in the differential count take place during the day? The question is important in view of the fact that in many of our experiments the leucocyte response took the form not so much of a hyperleucocytosis, as of a relative neutrophilia. It was therefore important to ascertain whether control animals exhibited such variations during a period of six to eight hours.

From a large series of estimations in control animals we shall quote the following to shew that any variation which occurs in the differential blood picture is exceedingly slight. The numbers are percentages :—

		21 Feb.			22 Feb.	23 Feb.
		10 a.m.	3'30 p.m.	5'30 p.m.	4 p.m.	11 a.m.
Rabbit I.	Small Lymphocytes	43	39	47	49	49
	Large Mononuclears	2	2	0	3	0
	Amphophiles	50	57	48	45	46
	Eosinophiles	0	0	0	0	0
	Mast cells	5	2	5	3	5
Rabbit II.		11 a.m.	5 p.m.			
	Small Lymphocytes	51	42			
	Large Mononuclears	0	0			
	Amphophiles	46	55			
	Eosinophiles	0	0			
Rabbit III.		10'45 a.m.	3 p.m.	5'30 p.m.		
	Small Lymphocytes	60	50	49		
	Large Mononuclears	0	0	1		
	Amphophiles	35	46	43		
	Eosinophiles	0	0	0		
Rabbit IV.		11'30 a.m.	3'15 p.m.	5'20 p.m.		
	Small Lymphocytes	47	43	52		
	Large Mononuclears	5	7	6		
	Amphophiles	40	39	35		
	Eosinophiles	0	0	0		
Rabbit V.		10 a.m.	1'30 p.m.			
	Small Lymphocytes	28	39			
	Large Mononuclears	14	9			
	Amphophiles	50	44			
	Eosinophiles	0	0			
Rabbit VI.		10'45 a.m.	1'45 p.m.	5 p.m.		
	Small Lymphocytes	34	21	36		
	Large Mononuclears	16	28	11		
	Amphophiles	46	48	54		
	Eosinophiles	0	0	0		
	Mast cells	4	3	9		

In the following experiment the control animal was inoculated intraperitoneally with 2 cc. of a 5 % solution of carbolic acid (*vide* Exp. 17):—

		24 Feb.			25 Feb.
		10'30 a.m.	3 p.m.	5'30 p.m.	11'30 a.m.
Rabbit VII.	Small Lymphocytes	39	23	21	32
	Large Mononuclears	2	0	0	0
	Amphophiles	56	75	77	64
	Eosinophiles	0	0	0	0
	Mast cells	3	2	2	4
	Total Leucocytes	5,950	5,500	5,900	5,400

It will be seen that in this last case a slight relative neutrophilia occurred, but in all the preceding cases the variations in the differential count throughout the day were quite inconsiderable.

GUINEA-PIG.

Much less attention has been paid to the leucocytes of the guinea-pig than to those of the rabbit.

Hirschfeld (*loc. cit.*) gave the following classification, but did not record any numerical data:—

- (1) Eosinophiles with large ball-shaped granules.
- (2) Indulinophiles with small ball-shaped granules.
- (3) Polynuclears without granulation (scarce).
- (4) Mast cells.
- (5) Large granulated lymphocytes.
- (6) Small non-granular lymphocytes.

Hirschfeld's indulinophile cells, whose granules take up the indulin from the eosin-aurantia-indulin mixture, correspond to the pseudo-eosinophile cells of Kurloff, and in function to the polynuclear cells of man.

Kurloff's (³⁷) classification was as follows:—

- (1) Polynuclear cells with pseudo-eosinophile granulation.
- (2) Typical eosinophile cells.
- (3) Nigrosinophile cells, differing from the true eosinophiles in this respect only, that, in the eosin-aurantia-nigrosin mixture, their granules take up the nigrosin tint while the eosinophile granules are red.

(4) Typical lymphocytes.

(5) The vacuolated large mononuclear cells. These latter cells were first noted by Kurloff in the blood of the guinea-pig. In their morphology, they correspond to the large mononuclear leucocytes of man. The protoplasm of these cells, however, is highly vacuolated, and included in the vacuole is a round or oval granular mass, staining a dull-red with Leishman's stain. It is impossible here to discuss the question of the nature of these bodies enclosed in the cytoplasm of the vacuolated cells. The whole subject has recently been treated in detail by one of us (Ledingham) ⁽⁴⁹⁾.

The following are the average counts (total and differential) recorded by Kurloff, Burnett ⁽³⁸⁾, compared with our own observations:—

	Burnett.	Kurloff.	Ledingham.
Small Lymphocytes	47	32	30
Large Mononuclears	10	17	21
Polynuclears	31	45	39
Eosins and Nigrosins	10	10	8
Mast cells	3	—	6
Total Leucocytes	10,897	12,600	12,312

In the control guinea-pigs, as in the control rabbits, the variations in the total and differential counts throughout the day were found to be quite trivial.

III.—Fluctuations in the opsonic index in healthy animals during short periods.

Does the opsonic index in healthy animals vary throughout the day or from day to day? To settle this important question numerous investigations were made, some of which we may here quote.

Opsonic indices of two healthy rabbits, C and E, on five successive days. (C taken as control.)

Date.	Opsonic Index.	
29/1/05	C = 1.0	E = 1.0
30/1/05	C = 1.0	E = 1.2
31/1/05	C = 1.0	E = .98
1/2/05	C = 1.0	E = .98
2/2/05	C = 1.0	E = .98

Opsonic indices at different times during the day :

	11'30 a.m.	3'15 p.m.	6'15 p.m.
Rabbit I.	1'0	'91	1'0
	10 a.m.	3 p.m.	
Rabbit II.	1'0	1'0	
	11 a.m.	5'20 p.m.	
Rabbit III.	1'0	'95	
	10 a.m.	3'30 p.m.	5'30 p.m.
Rabbit IV.	1'0	1'0	'94
	10'30 a.m.	3 p.m.	5'30 p.m.
Rabbit V.	1'0	'98	1'3
(N.B.—This rabbit received 2 cc. of a 5 % solution of carbolic acid. <i>Vide</i> Exp. 17.)			
	11'30 a.m.	3'15 p.m.	5'20 p.m.
Rabbit VI.	1'0	1'0	1'0
	11 a.m.	1 p.m.	
Guinea-pig I.	1'0	1'08	
	11'50 a.m.	3 p.m.	4'45 p.m.
Guinea-pig II.	1'0	1'2	1'07

From the above results it will be gathered that during periods from three to twenty-four hours the opsonic index shows very little variation. In fact, the figures 1'2 and '9 may be taken as the upper and lower limits of what one may either call "physiological opsonic variation" or more probably "experimental error."

IV.—Details of experimental results.

1.—*Sodium Cinnamate.*

In Charts I. to VII., are represented, graphically, the leucocytic and opsonic variations obtained by the inoculation of sodium cinnamate in rabbits and guinea-pigs.

The various leucocyte types are expressed in absolute values per cub. mm. The non-granular cells in which are included the small and large mononuclears and transitional cells are classed together for convenience, as it was invariably found that these cells reacted in the same direction to the chemical agents employed for inoculation.

In the following charts the polymorphonuclears are represented as "P," lymphocytes as "L," mast cells as "M," large mononuclears as "L M," and the eosin-nigrosinophiles as "E N." The total number of leucocytes is represented as "T." "ST" and "TB" denote that the opsonic power of the serum was tested against staphylococcus and tubercle bacillus respectively.

Regarding the granular series, our attention must be primarily directed towards the polymorphonuclear cells. Mast cell curves and eosinophile-nigrosinophile curves are also entered in the rabbit and guinea-pig experiments respectively.

The number of eosinophile cells in the rabbit, and of mast cells in the guinea-pig, is too small, and presents too slight variations to be conveniently charted. It has to be noted also that, in all cases, the inoculation was made either immediately or very shortly after the first sample of blood was drawn for purposes of total and differential count and estimation of opsonic content.

The quantity of sodium cinnamate (Merck) inoculated, varied from .5 to 1 gram.

Exp. I. (Chart I.) Rabbit. Sodium cinnamate 1 gram. dissolved in 10 cc. of sterile normal saline, inoculated intravenously.

A very considerable hyperleucocytosis (22,900) is apparent three to four hours after inoculation, and is also marked at the sixth hour. The rise was due entirely to the polynuclear series, the percentage of which rose from 49 before inoculation to 88 and 84 at the third and sixth hours afterwards, respectively, while the non-granular cells fell from an initial percentage of 40 to 10 and 14 at the same periods thereafter.

The mast cells also fell both relatively and absolutely.

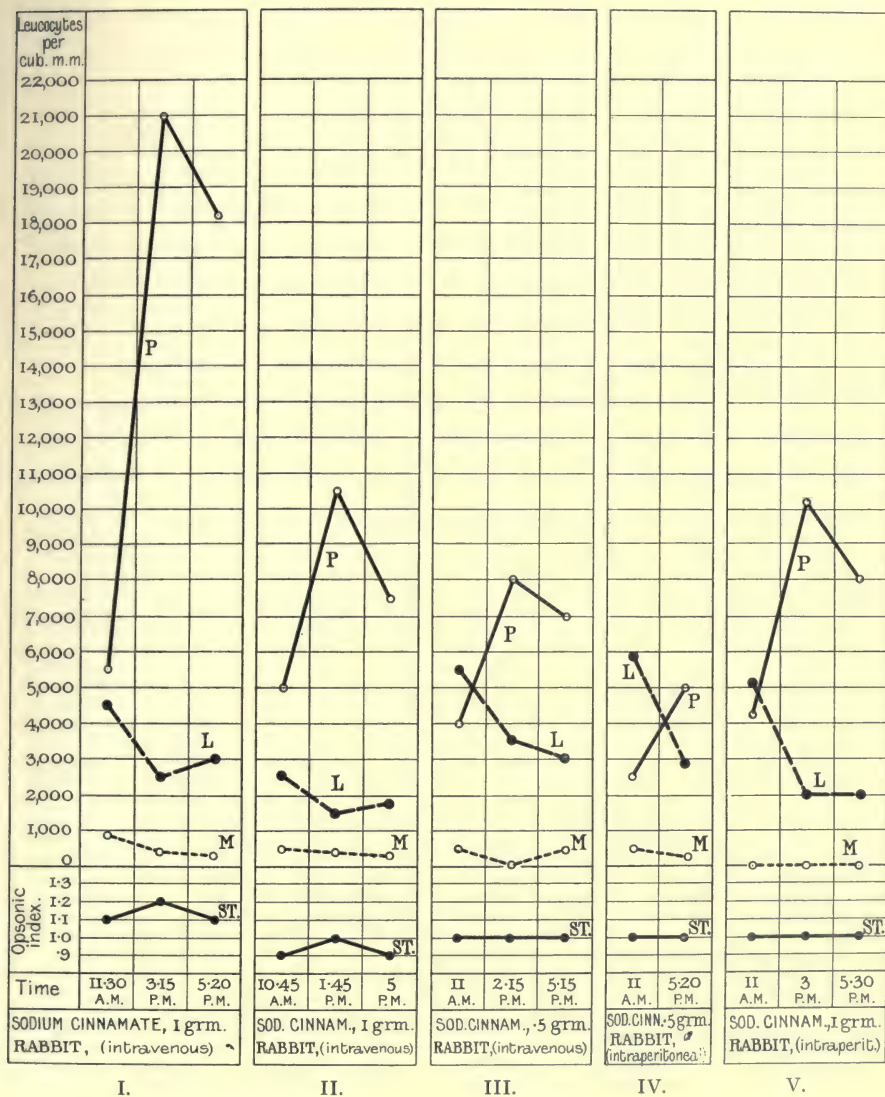
The opsonic variation during the period under consideration was scarcely appreciable.

Exp. II. (Chart II.) Rabbit. Intravenous inoculation.

The leucocytosis obtained was very slight at the third to the fourth hour after inoculation, and at the sixth hour the blood-picture had almost returned to the normal. The very considerable rise in the relative

percentage of polynuclear cells is striking, even in the absence of any great absolute rise.

The opsonic content showed practically no variation.



Exp. III. (Chart III.) Rabbit. Intravenous inoculation.

Half the usual quantity of sodium cinnamate was inoculated. No appreciable rise in the total count resulted, but the polynuclear percentage again rose from 34 to 69, while the non-granular and mast cells fell markedly.

No change took place in the opsonic content.

Exp. IV. (Chart IV.) Rabbit. Intraperitoneal inoculation.

In the peripheral circulation, no hyperleucocytosis took place, as has so frequently been observed after intraperitoneal inoculation, but the relative neutrophilia in the circulating blood is evidence of a possibly much greater quantitative leucocytic reaction in the peritoneal area.

No change took place in the opsonic content after six hours.

Exp. V. (Chart V.) Rabbit. Intraperitoneal inoculation.

A very slight absolute rise in the leucocyte count was evident in the peripheral circulation, but the relative neutrophilia was even more pronounced than in the last experiment. The percentage rose from 44 to 80 in four hours, and was still high (76 %) at the sixth hour.

No appreciable variation was noted in the opsonic content during the period of observation.

Exp. VI. (Chart VI.) Guinea-pig. Intraperitoneal inoculation
(.5 gm.).

In spite of the fact that the inoculation was intraperitoneal, an enormous rise of the total leucocytes in the peripheral circulation took place four hours after injection. The period of hyperleucocytosis was, however, very transitory, the count returning to its initial level at the 6th hour. The rise affected solely the polynuclear series. It is notable, also, that the relative neutrophilia persisted for some time after the hyperleucocytosis had disappeared.

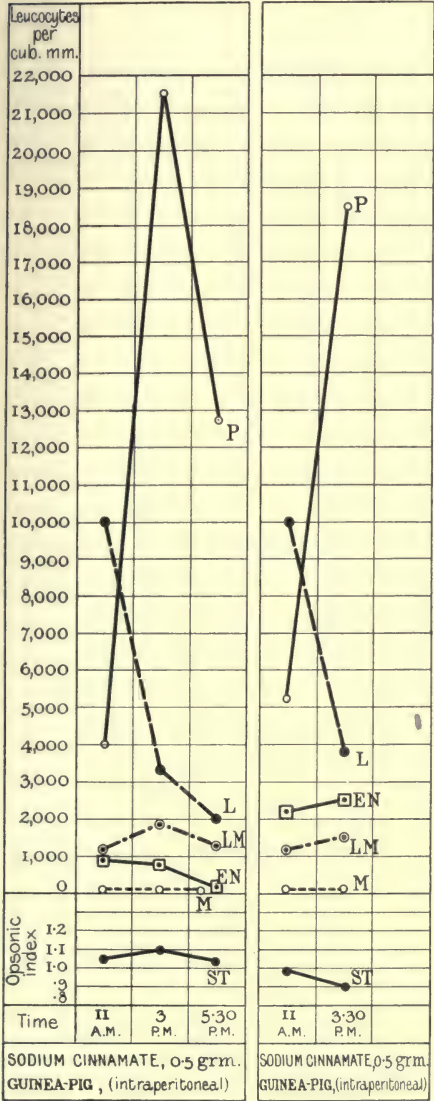
No variation took place in the opsonic content.

Exp. VII. (Chart VII.) Guinea-pig. Intraperitoneal inoculation.

The guinea-pig employed, presented a slight initial leucocytosis with, however, no derangement of the differential count, so that the figures, though high (19,200), must be taken as normal for that animal.

The total leucocytes rose in four hours to 27,200, and this rise was accompanied by a marked neutrophilia.

No opsonic variation was evident.



Remarks on Sodium Cinnamate
—Leucocytosis.

The intravenous inoculation of sodium cinnamate in rabbits produced the most marked hyperleucocytosis, while the intraperitoneal inoculation produced little or no rise in the total leucocytes of the peripheral circulation. It must be insisted, however, that the relative neutrophilia, which is a constant feature even in the absence of hyperleucocytosis, is as much an expression of leucocytic stimulation as the latter, and differs from it only in degree.

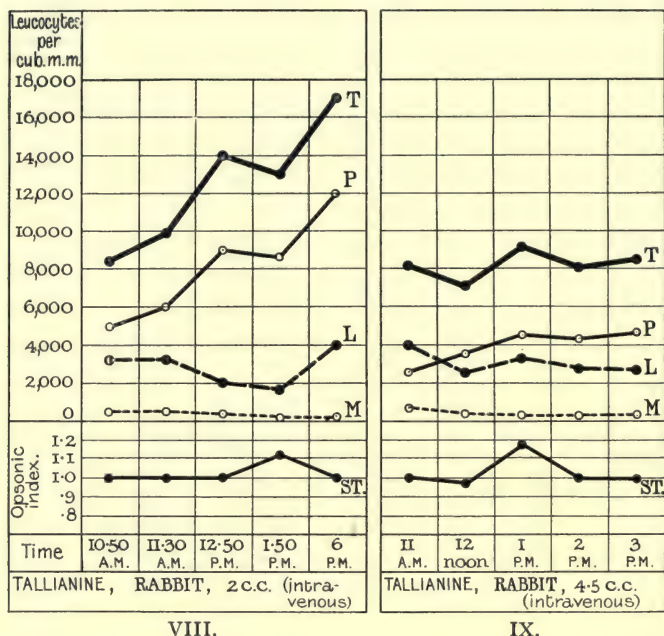
The duration of the period of hyperleucocytosis after a single inoculation was always short, in some cases lasting only a few hours. On examination, twenty-four hours after inoculation, the blood picture was invariably normal.

The results obtained by us, in the case of sodium cinnamate leucocytosis, are in entire accordance with those recorded by Richter⁽³⁹⁾, Richter and Spiro⁽⁴⁰⁾ and Batty Shaw⁽⁴¹⁾, and in entire disagreement with those of Charteris and Cathcart⁽⁴²⁾, who evidently missed the period of hyperleucocytosis entirely, by

taking their blood samples at 24-hour intervals. The assertion of these latter observers that the leucocytosis produced is a mononuclear one is evidently incorrect, and due to the same cause.

2.—*Tallianine*.

This substance, recently introduced by Stassano and Billon ⁽⁴³⁾, is formed by the action of ozone on essence of turpentine, the action being stopped when there has been absorption of a quantity corresponding to four volumes of ozone. Tallianine, which is supplied by Brignonet et



Gaubert (Paris),* in sealed tubes, is a clear watery fluid with a faint terebene odour.

Exp. VIII. (Chart VIII.) Rabbit. Intravenous inoculation of 2 cc. of Tallianine.

In view of the fact that Stassano and Billon, in their brief note on the subject, asserted that the leucocytosis occurs very quickly after

* BRIGNONNET père et fils et GAUBERT, 15 rue du Landy. Plaine St. Denis (Seine).

inoculation, and that its duration is always transitory, we made complete

blood and opsonic estimations every hour during the first three or four hours.

Blood drawn 40 minutes after injection showed practically no change either in the absolute or differential count. Two hours after inoculation, however, the total leucocytes had risen from 8,700 to 14,500, and there was a marked relative neutrophilia. At the third hour the neutrophilia had still further increased, though the total count had slightly fallen. A final estimation at the seventh hour showed that, in the interval, the leucocytes had again mounted upwards to 17,000, while the neutrophilia was well maintained. During the seven hours that the animal was under observation, no change at all took place in the opsonic content of the serum.

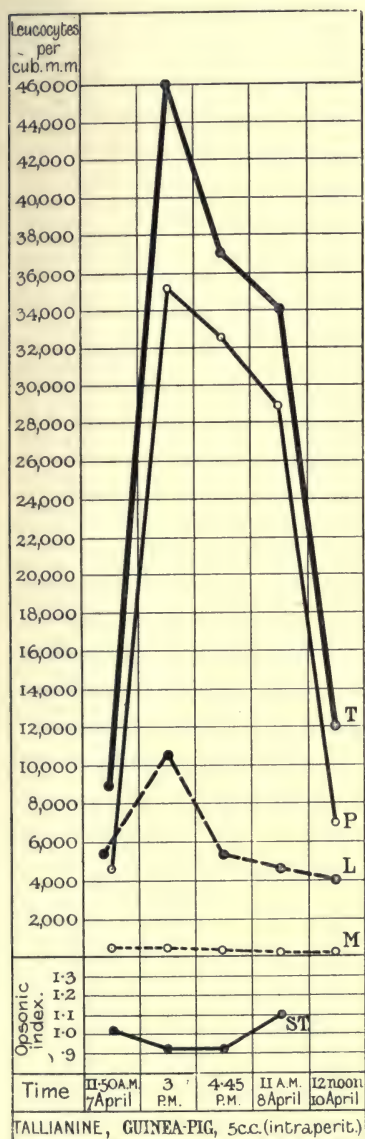
Exp. IX. (Chart IX.) Rabbit. Intravenous inoculation (4.5 cc.).

Hourly examinations were made after inoculation, but practically no change took place in the blood-picture either in the direction of hyperleucocytosis or relative neutrophilia.

The fluctuations in the opsonic content were also trivial during the period of observation.

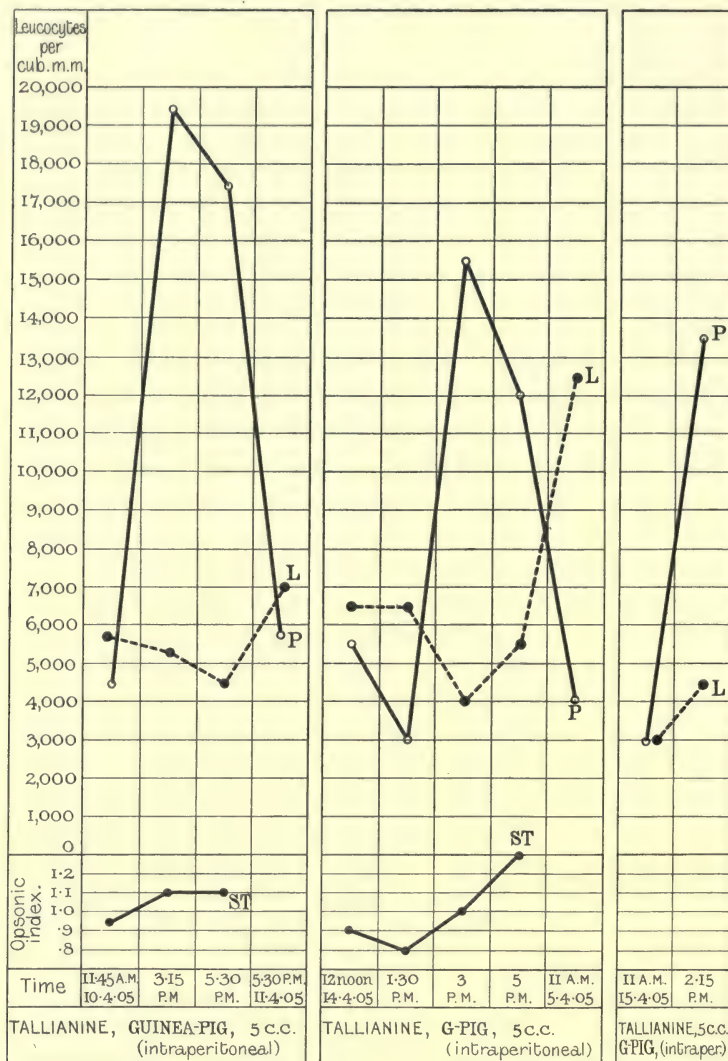
Exp. X. (Chart X.) Guinea-pig. Intraperitoneal inoculation of 5 cc. of Tallianine.

Three hours after inoculation, an enormous rise had taken place in (341)



X.

the total leucocytes (46,300). The rise affected solely the polynuclears. At the fifth hour the hyperleucocytosis and neutrophilia were maintained,



XI.

XII.

XIII.

and even at the end of 24 hours very little change had taken place.

Unfortunately, no count was made at the end of 48 hours, but, at the end of 72 hours, there was still a very slight relative neutrophilia, although the total count had returned to practically normal limits.

Estimations of the opsonic content during the first 24 hours revealed no change either towards tubercle bacilli or staphylococci.

Exp. XI. (Chart XI.) Guinea-pig. Intraperitoneal inoculation
(5 cc. of Tallianine).

In this animal a fairly well-marked hyperleucocytosis and neutrophilia were again registered, but the duration was much shorter, the blood-picture returning to the normal at the end of 24 hours.

The opsonic content during the period of hyperleucocytosis was not appreciably affected.

Exp. XII. (Chart XII.) Guinea-pig. Intraperitoneal inoculation
(5 cc. of Tallianine).

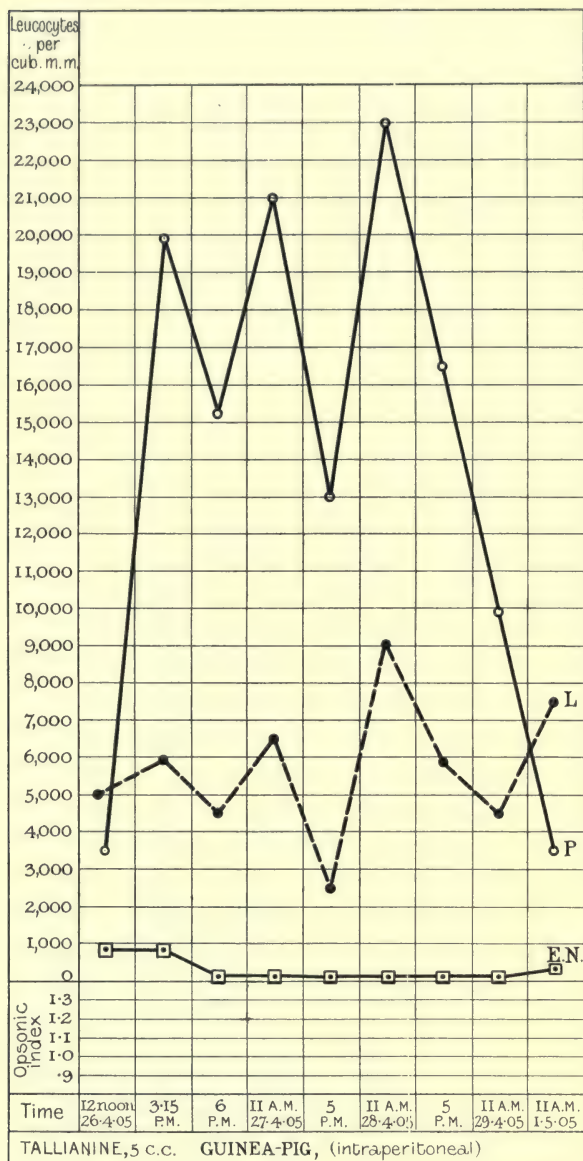
On this occasion, a slight leucopenia was apparent at the end of the first hour, the fall affecting mostly the polynuclears. At the end of the third hour, however, a very marked hyperleucocytosis and neutrophilia were evident. At the fifth hour this condition was still present though the neutrophilia had slightly diminished. At the end of 24 hours there was still a well marked hyperleucocytosis, but the neutrophilia had entirely disappeared. A late response on the part of the mononuclears was now evident. The opsonic content, during the observation period, presented only slight fluctuations.

Exp. XIII. (Chart XIII.) Guinea-pig. Intraperitoneal inoculation
(5 cc. of Tallianine).

At the end of three hours, the leucocytes had mounted from 7000 to 19,800, and relative neutrophilia was conspicuous. The opsonic content was not estimated in this experiment, advantage being taken of the hyperleucocytosis, to test the phagocytic power of the newly-formed polynuclears (*vide infra*).

Exp. XIV. (Chart XIV.) Guinea-pig. Intraperitoneal inoculation
(5 cc.).

In this experiment, the animal was kept under observation for six days after inoculation, and blood examinations were made every day.



XIV.

Three hours after inoculation, the leucocytes had risen to 26,600, and the polynuclear percentage from 37 to 74.

At the sixth hour, the total count was still high (20,000), and the neutrophilia maintained.

At the end of 24 hours a count of 28,100 was registered, with a corresponding neutrophilia.

At the end of 29 hours the count fell to 17,600, while the neutrophilia increased.

At the end of 48 hours the total leucocytes had again risen to their highest level during the experiment (33,000).

At the end of 53 hours the total had fallen slightly, with little change in the neutrophilia.

At the end of 72 hours the count had fallen to 14,500, and the neutrophilia showed signs of disappearing.

The final estimation was made at the end of 120 hours, when the leucocytes were found to have fallen to the normal level, and the neutrophilia had completely disappeared.

The long duration of the period of hyperleucocytosis in this experiment is remarkable, and, taken in conjunction with the previous experiments, would tend to show that in the guinea-pig, at least, the effect of the tallianine on the hæmopoietic organs is powerful and prolonged.

Remarks on Tallianine—Leucocytosis.

The leucocytosis due to tallianine is variable, and may be entirely absent when intravenous injection in rabbits is employed.

Intraperitoneal inoculation in guinea-pigs invariably resulted in marked hyperleucocytosis, which might persist over three or four days.

Neutrophilia is invariably a conspicuous feature. We did not find so rapid a response to tallianine, on the part of the white cells, as did Stasano and Billon. As a rule the response did not make itself apparent before the second or third hour.

No injurious effects on the animals in question were ever noted.

In one rabbit experiment (intravenous inoculation of 5 cc.), not charted, there resulted no change whatever either in the direction of hyperleucocytosis or relative neutrophilia, though estimations were made hourly for six hours after inoculation. It would seem that in the rabbit, at least, tallianine must disappear very rapidly from the circulation after intravenous inoculation, while the absorption proceeds more slowly from the guinea-pig's peritoneum.

3.—*Bouillon.*

Bouillon has long been known to produce a local leucocytosis when injected into the peritoneal cavity. (Pfeiffer and Issaëff (*loc. cit.*) and Funck (⁴⁴)).

Exp. XV. (Chart XV.). Rabbit. Intraperitoneal injection of 10 cc. of a 1 % sterile peptonised bouillon.

Before the inoculation, the animal was found to have a very low leucocyte count (4,500), but four hours afterwards it had risen to 7,350,

(345)

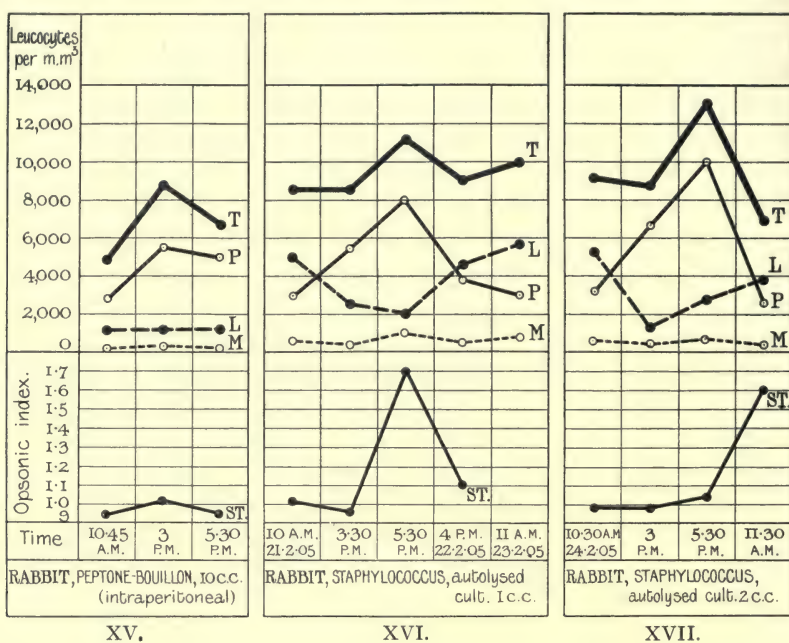
and there was a fairly well-marked neutrophilia which was maintained at the seventh hour after inoculation.

During the period of observation no rise took place in the opsonic content.

4.—*Filtrates from autolysed staphylococcus cultures.*

Exp. XVI. (Chart XVI.) Rabbit. Intraperitoneal injection of 1 cc. of autolysed staphylococcus culture (= filtrate from 70 million cocci).

Five hours after injection there was no rise in the total count, but there was a marked relative neutrophilia. At the seventh hour, there was



a slight rise in the total count and a still more marked neutrophilia. At the end of 24 hours, the polynuclears had fallen to a percentage of 42, and at the end of 48 hours they had returned to their initial level.

Regarding the opsonic content, no change took place in the first five hours, but, at the seventh hour, the opsonic index rose to 1.7. The high opsonic tide was only of short duration, and had completely disappeared at the end of 24 hours.

Exp. XVII. (Chart XVII.) Rabbit. Autolysed staphylococcus culture (2 cc.)

A relative neutrophilia was apparent at the end of four hours, but no hyperleucocytosis. At the end of $6\frac{1}{2}$ hours there was a very moderate hyperleucocytosis of neutrophilia type, which had completely disappeared at the end of 24 hours. On this occasion the opsonic index did not rise at all during the first seven hours, but a sample taken at the end of 24 hours showed a fairly high opsonic index (1.6).

5.—*Pilocarpine*.

Ruzicka (*loc. cit.*) states that the immediate effect of an intravenous injection of pilocarpine, in doses up to 75 mg., in the rabbit, is the production of an enormous leucocytosis. In one case the leucocytes rose to 235,000 almost immediately after inoculation of 50 mg. intravenously. He lays stress on the fact that the hyperleucocytosis is never preceded by a period of hypoleucocytosis.

Ruzicka's results have not been generally confirmed. Spiro ⁽⁴⁵⁾ found an initial leucopenia with pilocarpine, followed later by a period of hyperleucocytosis of neutrophile type. In the two following experiments, this phenomenon was strikingly brought out.

Exp. XVIII. (Chart XVIII.) Rabbit. 60 mg. injected subcutaneously.

Ten minutes after injection the leucocytes fell from 13,400 to 10,900, and one hour afterwards to 9,100. The count then commenced to rise. Three hours after inoculation, the leucocytes had reached their initial level, and at the sixth hour, a marked hyperleucocytosis of neutrophile type was evident.

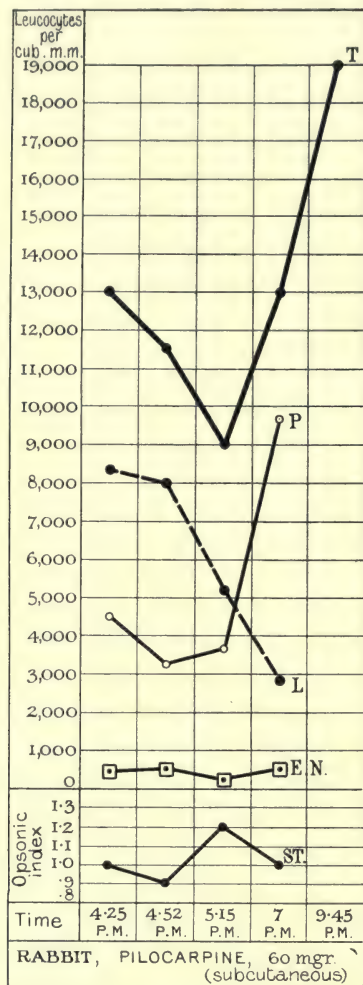
A slight rise in the percentage of the lymphocytes during the first half-hour was the only evidence of an initial mononucleosis.

During the experiment, no appreciable change in the opsonic content took place.

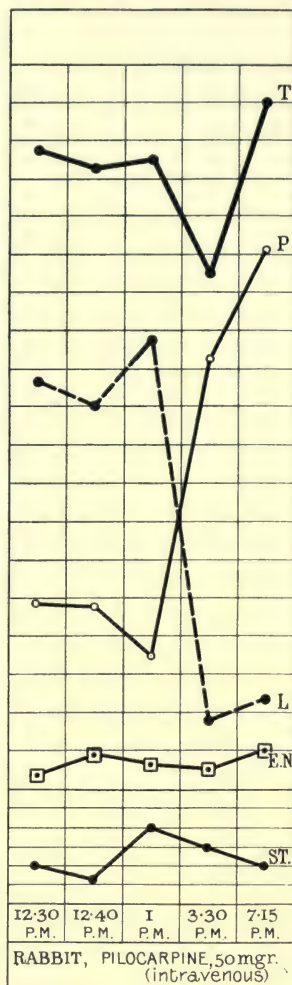
Exp. XIX. (Chart XIX.) Rabbit. Intravenous inoculation (50 mg.).

Ten minutes after injection practically no change had taken place either in the total or differential count. During the next twenty minutes,

however, a marked relative mononucleosis was evident with little absolute change. In the course of the next two-and-a-half hours the total count fell slightly, but this was accompanied by a very marked neutrophilia, the



XVIII.



XIX.

percentage rising from 22 to 84. At the end of the next four hours, *i.e.*, eight hours after injection, hyperleucocytosis, with relative neutrophilia, was well established.

The fluctuations in the opsonic content during the experiment were quite trivial.

In similar experiments, not here charted, we never succeeded in obtaining a marked hyperleucocytosis immediately after intravenous injection as Ruzicka did. In fact, the hyperleucocytosis appears to be a late phenomenon.

In the foregoing experiments, chemical substances of more or less simple chemical constitution have been employed, to produce artificial leucocytosis. We have seen that in the presence of marked hyperleucocytosis there has been no corresponding rise in the opsonic content.

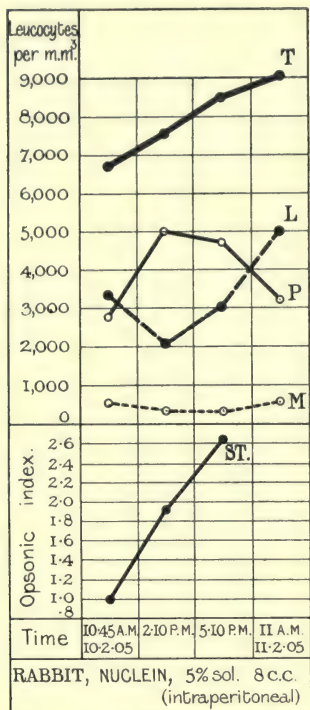
6.—*Nuclein.*

We have already referred to the employment of nuclein by Issaëff (*loc. cit.*) in experimental cholera infection. Similar results were obtained by Miyake ⁽⁴⁶⁾ working in Mikulicz's laboratory, on experimental coli infection in guinea-pigs. By preliminary intraperitoneal inoculation of nuclein, he was able to increase enormously the resistance of the peritoneum towards a subsequent injection of virulent coli, made during the period of hyperleucocytosis. The same principle was adopted by Mikulicz in surgical cases in man, involving laparotomy, and it was found that previous injection of nuclein acted as a good safeguard against a possible subsequent infection. He stated that a hyperleucocytosis in the circulating blood regularly followed the injection of nuclein in man. The stage of hyperleucocytosis was usually preceded in animals by a period of hypoleucocytosis occurring during the first two hours after inoculation.

Recently, Huggard and Morland ⁽⁴⁷⁾ have tried the effect of yeast administration on the opsonic index in tuberculous patients. The dose was usually 10 grm. of ordinary German yeast, and was administered for at least a month in each case. In a series of 25 unselected cases, they found a mean rise in the opsonic index of '15 after a mean course of 41 days' treatment.

Two days after the administration of a single dose there was a slight fall in the opsonic index, followed after four or five days by a rise. The opsonic variations recorded by these authors are, however, very slight, and might well come within the experimental error. Thus, after a dose

of yeast, there might be a fall in the opsonic index, from .8 to .7, with a subsequent rise to .9. Marked changes also occurred in the leucocyte count in certain cases. During the first half-hour after a dose of 10 grm. the leucocytes were more than doubled. In the second half-hour there was a fall equal to half the previous rise. In three hours the leucocytes



XX.

sank below the initial level. It has to be noted, also, that some of the patients were taking drugs like benzsol quinine, terpin hydrate, calcium glycerophosphate, etc., along with the yeast, so that the pure effect of the latter must have been somewhat masked. It seems highly probable, however, from our nuclein experiments presently to be detailed, that the leucocytic and opsonic response (though the latter was undoubtedly very slight) were attributable to the nuclein contained in the yeast.

Exp. XX. (Chart XX.) Rabbit. Intraperitoneal injection of 8 cc. of a 5 % solution of Nuclein.*

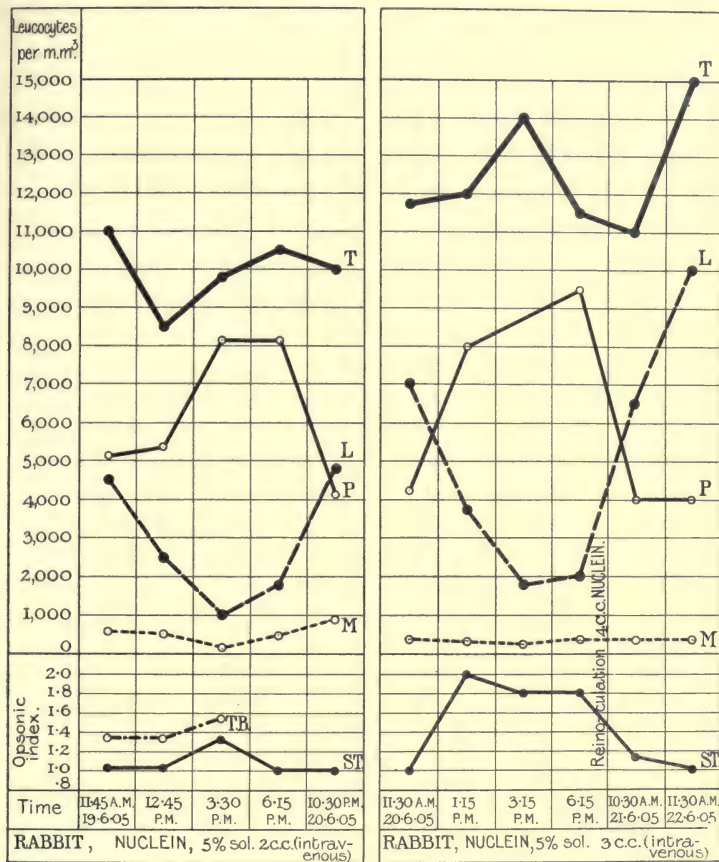
During the first six hours very little variation took place in the leucocyte count apart from a slight neutrophilia. The opsonic index, however, reached a level of 1.9 at the end of three hours, and of 2.6 at the end of 5½ hours.

Exp. XXI. (Chart XXI.) Rabbit. Intravenous inoculation (2 cc.).

On this occasion, frequent samples were taken with a view to the demonstration of a negative phase. Very little change took place in the leucocyte count apart from a transient neutrophilia and a slight leucopenia following inoculation.

* The nuclein used in the experiments was Nuclein Solution No. 1, containing 5 % Nucleinic acid (from yeast), manufactured by Parke, Davis & Co.

The opsonic index to staphylococcus did not rise very high on this occasion (1.38). At the fourth hour, the opsonic index to tubercle rose to 1.5, but at the end of 24 hours had returned to unity.



XXI.

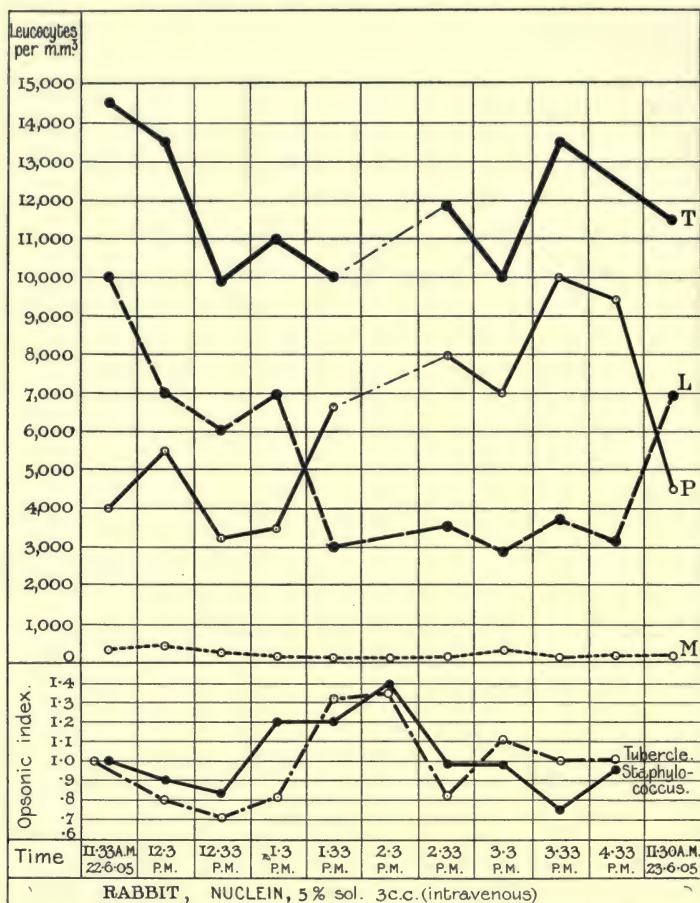
XXII.

Exp. XXII. (Chart XXII.) Rabbit. Intravenous inoculation (3 cc.).

A slight hyperleucocytosis, with a very well-marked neutrophilia, appeared four hours after inoculation. At the seventh hour, the hyperleucocytosis had gone, but the neutrophilia persisted. Twenty-four hours after inoculation, the blood presented the initial leucocytic formula.

The opsonic variation was very remarkable on this occasion. Less

than two hours after inoculation, the opsonic power had doubled its original value, and, at the seventh hour, it was still very high (1·8). At this point a further inoculation of 4 cc. was given intravenously, but any cumulative effect that may have occurred had quite disappeared at the end of 24 hours after the first injection.



XXIII.

Exp. XXIII. (Chart XXIII.) Rabbit. Intravenous inoculation (3 cc.).

Samples were drawn on this occasion every half-hour in the hope of demonstrating a leucocytic or opsonic negative phase.

During the first hour there was little change in the leucocytic formula, although the total count diminished.

During the second hour, while the leucopenia persisted, the polynuclear percentage rose considerably (69.5). At the end of the third hour it had reached 70, where it remained till the end of the fifth hour.

The total count during the latter period varied slightly, but there was no distinct period of hyperleucocytosis. At the end of 24 hours the leucocytic formula had returned to the normal.

The opsonic index exhibited a marked negative phase during the first hour after inoculation, and was succeeded by a moderate rise during the second hour. The maximum opsonic power, both to staphylococcus and tubercle, was registered in the course of the third hour, but was exceedingly transitory. It fell to normal again in the fourth and fifth hours.

The negative phase in the case of tubercle was somewhat more pronounced than in the case of staphylococcus. It is notable that the high opsonic level coincided fairly exactly with the attainment of the high neutrophilic phase, but this latter persisted much longer.

Exp. XXIV. (Chart XXIV.) Guinea-pig. Intraperitoneal inoculation (1 cc.).

A well marked hyperleucocytosis of neutrophilic type took place, and the opsonic index rose to double its original value in four hours.

Exp. XXV. (Chart XXV.) Rabbit. (1 cc.).

Four hours after inoculation, the leucocytes showed a very slight rise, which was accompanied by an enormous neutrophilia (80 %). The opsonic index to staphylococcus also rose in four hours to 1.6.

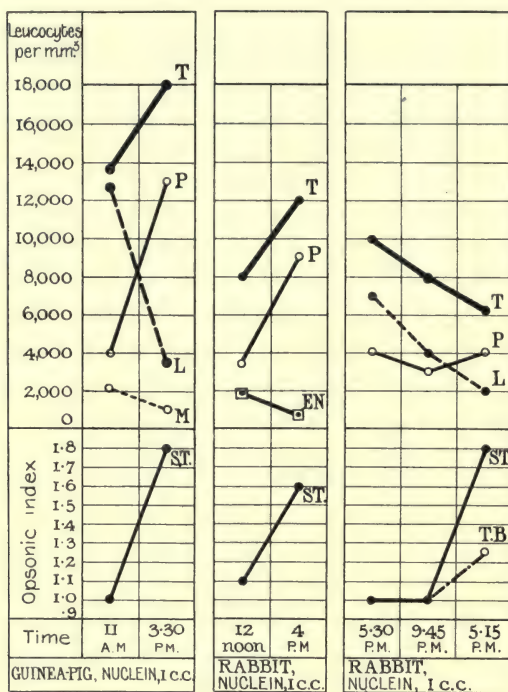
Exp. XXVI. (Chart XXVI.) Rabbit. (1 cc.).

The inoculation was made at 5.30 p.m., but by next morning, any effect that the nuclein may have had on the leucocytes and opsonic content had completely disappeared. Accordingly, another inoculation was given at 10.45 a.m. A sample drawn at 5.15 p.m. on the same day

showed a slight neutrophilia, but a very marked rise in the opsonic index, both towards staphylococcus and tubercle (1·8 and 1·31 respectively).

Exp. XXVII. (Chart XXVII.) Rabbit. Intravenous inoculation (2 cc.).

The opsonic power was tested both towards staphylococcus and tubercle. A marked rise took place at the end of five hours, but at the end of 24 hours the opsonic index had returned to normal.



XXIV.

XXV.

XXVI.

Exps. XXVIII. and XXIX. (Charts XXVIII. and XXIX.).

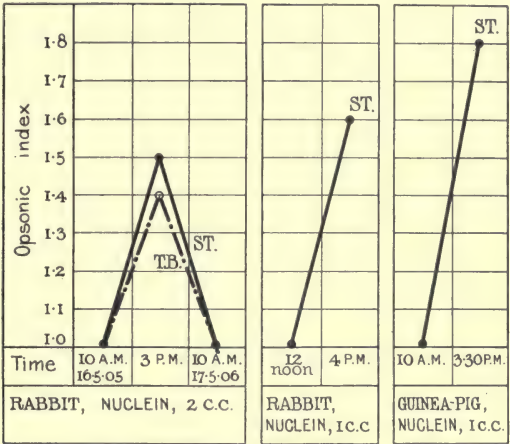
The opsonic estimations are referred to under Exps. XXIV. and XXV., but are re-charted alongside Chart XXVII., to show the varying degrees of nuclein effect on the opsonic power.

Exp. XXX. (Chart XXX.) Guinea-pig. Intraperitoneal inoculation (4 cc.).

Complete blood and opsonic estimations were made every hour after injection. At the end of the first hour, the total leucocytes had fallen slightly, but a relative neutrophilia was already conspicuous (70 %).

At the end of the second hour, the leucocytes had risen to 14,900, and an enormous neutrophilia was present (90 %).

At the end of the third hour, the neutrophilia was still high, though the total leucocytes had fallen.



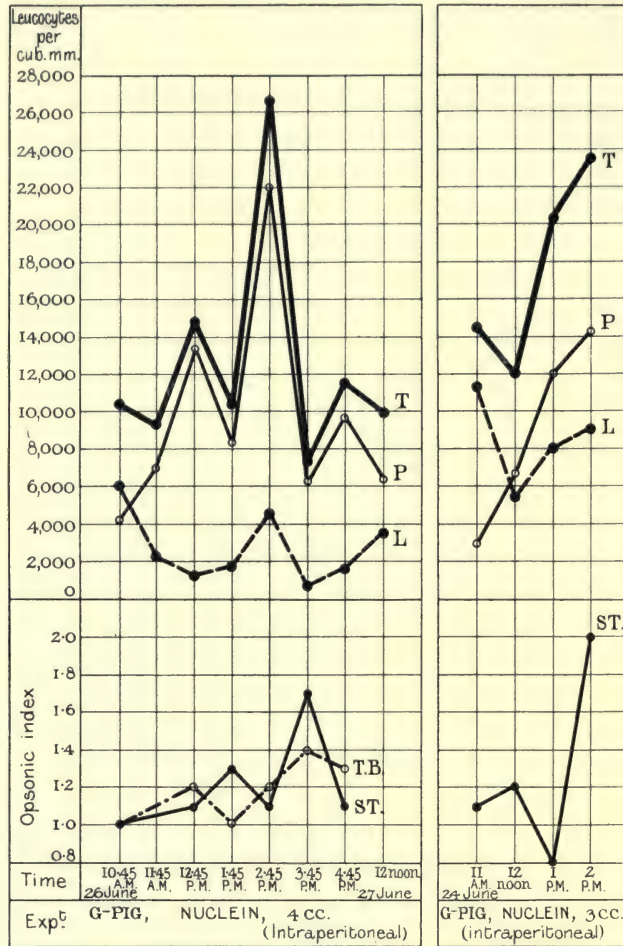
During the next hour a well marked hyperleucocytosis occurred (26,600) which had quite gone in the course of an hour. The neutrophilia was persistent.

A very marked rise in the opsonic index, both to staphylococcus and tubercle, took place between the fourth and fifth hours after inoculation. In both cases the rise was very transitory. There was no apparent negative phase. It is notable also that the relative neutrophilia reached its maximum two hours before the period of hyperleucocytosis.

Exp. XXXI. (Chart XXXI.) Guinea-pig. Intraperitoneal inoculation (3 cc.).

At the end of the second hour, there was a marked hyperleucocytosis with very slight relative neutrophilia. The opsonic index was double its

original value at the end of the third hour, thus coinciding with the maximum of hyperleucocytosis.



XXX.

XXXI.

Remarks on the effects of the injection of Nuclein.

It will be seen that the leucocytoses produced by nuclein have never been marked, and that in some cases a distinct leucopenia has resulted.

In spite of the slight absolute changes, however, the relative changes in the direction of neutrophilia have been quite conspicuous, though more transitory than in the case of tallianine.

The leucocytes of the guinea-pig react much more vigorously to nuclein than those of the rabbit.

In sharp contradistinction to substances like sodium cinnamate and tallianine, nuclein invariably produces a marked rise in the opsonic index, probably of a non-specific nature.

An experiment was made to determine whether the direct addition of nuclein to normal serum altered the opsonic index of the latter. The result was negative.

				Cocci per Leucocyte.
1.	Rabbit's serum	1 vol. + Salt sol.	1 vol. + Cocci + Leucocytes	= 6.9
2.	"	" + Nuclein sol. (50 %)	1 vol. + " + "	= 6.7
3.	"	" + Nuclein-salt sol. (1 in 2)	1 vol. + " + "	= 7.2
4.	"	" + Nuclein-salt sol. (1 in 4)	1 vol. + " + "	= 6.5
5.	"	" + Nuclein-salt sol. (1 in 8)	1 vol. + " + "	= 7.0
6.	"	" + Nuclein-salt sol. (1 in 16)	1 vol. + " + "	= 6.6
7.	"	" + Nuclein-salt sol. (1 in 32)	1 vol. + " + "	= 6.8
8.	"	" + Nuclein-salt sol. (1 in 64)	1 vol. + " + "	= 6.4

7.—*Papayotin-albumose.*

The papayotin-albumose was prepared by digesting washed fibrin with papayotin at 37° C for two days, filtering and precipitating the albumose of the filtrate by alcohol. The resulting precipitate was then dried over H₂ SO₄ in vacuo. Intraperitoneal injection of ½ gm. of this substance in rabbits produced a marked relative neutrophilia, but did not affect the opsonic index.

8.—*Aleurone Emulsion.*

The following experiment was made with aleuronat emulsion.*

A rabbit was inoculated intraperitoneally with 5 cc. of a 5 % emulsion of aleuronat. At the end of 24 hours the opsonic power of the serum was unchanged. At the end of 36 hours the animal was killed. On opening the abdomen a yellowish cheesy mass was found, adherent to the parietal peritoneum. It weighed 1.5 gm., and was composed of

* The aleurone used was aleuronat purissimum, prepared by R. Hundhausen. Hamm, i. W.

aleurion granules and leucocytes, a differential count of which showed, polynuclears 62 %, lymphocytes 22 %. The mass was frozen and thawed according to Buchner's method, mixed with an equal weight of .85 % NaCl., and ground with sand in a mortar. It was then centrifuged. The opsonic power of the fluid was then tested along with the serum of the same animal and the serum of a control animal.

The result was as follows :—

Control serum, 5 cocci per leucocyte.

Serum of aleurionat rabbit, 4 cocci per leucocyte.

Aleurionat exudate of rabbit, 0 cocci per leucocyte.

No opsonin, therefore, was present in the aleurionat exudate in this experiment, but without further experiments of a similar nature it would be unsafe to generalise as to the opsonic content of these leucocytic exudates.

9.—*Albumen-metaphosphate.*

In view of the fact that the opsonic index was so markedly influenced by nuclein, it was considered of interest to determine whether the so-called pseudo-nucleins, formed by precipitating albumen solutions with metaphosphoric acid, would produce analogous effects. The method employed for the preparation of the substance was one of the many recommended by Fuld (⁴⁸). To a certain volume of a 10 % solution of egg albumen, an equal volume of a 10 % solution of metaphosphoric acid was added. After filtration the precipitate was suspended in distilled water, and finally centrifuged. The process was repeated for five or six times. The precipitate of albumen-metaphosphate was then dried over sulphuric acid *in vacuo*, and finely powdered. Of this substance .5 gm., dissolved in 2 % sodium carbonate, was inoculated intraperitoneally in guinea-pigs and 1 gm. in rabbits.

From a long series of experiments we may quote the following, to show that no very marked changes took place in the opsonic index :—

Exp. I. Guinea-pig. (.5 gm. intraperitoneally at 3 p.m.).

	1st day,					2nd day,	
	3 p.m.	4 p.m.	5 p.m.	6 p.m.	11 p.m.	11 a.m.	11 p.m.
Opsonic Index.	1.0	.84	.7	.64	1.4	1.3	1.2

(358)

Exp. II. Rabbit. (.5 gm. intraperitoneally at 1'15 p.m.).

	1'15 p.m.	2'30 p.m.	3'30 p.m.	5 p.m.	9 p.m.
Opsonic Index.	1'0	'85	1'1	'96	'77

A notable feature was a rather prolonged negative phase (as in Exp. I.), which was not, however, followed by a high opsonic tide.

V.—On the reduced phagocytic power of the neutrophile leucocytes during the period of hyperleucocytosis.

We have seen that, under conditions, in which the polynuclear leucocytes are greatly in excess in the blood stream, there may be no appreciable change in the opsonic content (sod. cinnamate and tallianine experiments) as measured by normal leucocytes.

It seemed to us, therefore, a question of importance to determine whether the opsonic index would be altered by employing leucocytes drawn during the period of hyperleucocytosis as phagocytes in the *in vitro* test. The results of a series of experiments in this connection will be found on the next page.

It will be seen that the employment of the newly-formed polynuclear leucocytes, as phagocytes in the opsonic test, invariably lowers the opsonic index, and sometimes very considerably. The new leucocytes, in fact, appear to have their phagocytic powers very feebly developed. Now, it is obvious that, what one may call the "real opsonic index," must be measured by the serum and leucocytes of the same animal taken at the same time. In those cases, therefore, where the opsonic content of the serum remains fairly constant (sod. cinnamate and tallianine), the real opsonic index will be very much lowered during the period of hyperleucocytosis or of relative neutrophilia. Again, in experiments with nuclein and autolysed cultures, the real opsonic index will still be much higher than in the control (*vide* Exps. III. and IV., Table), but not so high as when normal leucocytes are employed in the test. The above results have obviously a bearing on the question of vaccine-therapy in man, where possible variations in the phagocytic power of the leucocytes during vaccinal leucocytosis may influence considerably the real opsonic index. Any increase in the opsonic content of the serum might be neutralised, or rendered unavailable to the organism, by deficient phagocytic power on the part of the leucocytes.

Experiments demonstrating the Reduced Phagocytic Power of the Neutrophilic Leucocytes during the period of Hyperleucocytosis.

No.	Experiment.	Animal.	Hyper-leucocytosis.	Relative Neutrophilia.	Leucocyte count at time of sample.	Scheme of Experiment.	Cocci per leucocyte.	T.B. phagocyte.
1.	Sodium Cinnamate.	Rabbit.	Slight.	Marked.	12,300	Control serum + control leucocytes. " " + cinnamate " Cinnamate " + control " " " + cinnamate "	4.7 1.3 2.2 0.8	
2.	Sodium Cinnamate.	Guinea-pig	Marked.	Marked.	27,200	Control serum + control leucocytes. " " + cinnamate " Cinnamate " + control " " " + cinnamate "	12.0 6.0 11.0 7.8	
3.	Nuclein Solution.	Rabbit.	Slight.	Marked.	11,100	Control serum + control leucocytes. " " + nuclein " Nuclein " + control " " " + nuclein "	6.7 3.0 8.6 5.6	
4.	Nuclein Solution.	Guinea-pig	Moderate	Marked.	18,000	Control serum + control leucocytes. " " + nuclein " Nuclein " + control " " " + nuclein "	9.8 5.6 not tested 7.6	
5.	Tallianine.	Guinea-pig	Moderate	Marked.	17,400	Control serum + control leucocytes. " " + tallianine " Tallianine " + control " " " + tallianine " Rabbit's " + control " " " + tallianine " 3.7 3.0 4.8 2.9 2.5 0.8	
6.	Tallianine.	Guinea-pig	Marked.	Marked.	Three samples. Before inoc'n. (10,200) = P1 3.15 (24,800) = P2. 5.30 (21,400) = P3.	Tallianine serum (3.15) + P1 leucocytes " " + P2 " Control " + P1 " " " + P2 " Tallianine " (5.30) + P1 " " " + P3 " Control " + P3 "	14.5 9.9 18.6 11.4 17.8 8.0 4.2	
7.	Tallianine.	Guinea-pig	Marked.	Moderate	Two samples. Before inoc'n (7000) = P1. 2.15 p.m. (19,800) = P2.	Tallianine serum + P1 leucocytes. " " + P2 " Control " + P1 " " " + P2 " 6.0 3.3 6.8 3.8	
8.	Tallianine.	Guinea-pig	Marked.	Marked.	Two samples. Before inoc'n (9500) = P1. 3.13 p.m. (26,600) = P2.	Tallianine serum + P1 leucocytes. " " + P2 " Control " (a) + P1 " " (a) + P2 " Control " (b) + P1 " " (b) + P2 "	14.2 13.9 18.0 11.9 18.0 12.7	
9.	Tallianine.	Guinea-pig	None.	Slight.	11,600	Control serum + control leucocytes. " " + tallianine " Tallianine " + control " " " + tallianine "	11.2 10.0 10.6 8.5	

VI.—Summary and Conclusions.

1. The opsonic content is uninfluenced by the presence of hyperleucocytosis, or relative neutrophilia in the circulating blood, when these conditions are brought about by the injection of simply-constituted chemical bodies, like sodium cinnamate, tallianine, pilocarpine, etc.

2. The opsonic content is very markedly influenced by the inoculation of substances like nuclein, autolysed cultures, etc., the chemical constitution of which is exceedingly complex, and probably closely akin to that of the bacterial proteines. These substances at the same time produce decided changes in the blood picture, though these are, as a rule, less marked than in the case of the simpler bodies above mentioned.

3. During the period of hyperleucocytosis, the phagocytic power of the neutrophile leucocytes is considerably reduced.

Naturally, we have considered whether any conclusions can be drawn from the above-mentioned experiments, in reference to the important questions of the origin, and probable nature, of the opsonic substances in the serum.

In the first place, it is improbable that the substance is of the nature of complement, otherwise one might have expected an increase of the opsonic content of the serum, after the inoculation of substances like sodium cinnamate, which are known to raise the hæmolytic complement content concurrently with the production of a neutrophilic leucocytosis.

On the assumption that the opsonin is of the nature of an intermediary body which manifests its full action when co-operating with a complement, the results might be more readily explained. Thus, even with complement-increasing substances, like sodium cinnamate, it might be supposed that there is always just sufficient normal complement to neutralise the available amboceptor, and hence no opsonic rise would be recorded. The injection of substances like nuclein, of highly complex chemical constitution, and possibly allied to bacterial proteins, might produce an increase of intermediary body. The effect on the leucocytes might at the same time produce an increase of complement, and thus the opsonic content might rise. A third possibility is that the opsonin is a

simple substance, which is formed after inoculation of nuclein and bacterial proteins.

The experiments we have detailed above do not, however, admit of a solution of these problems, which, most likely, must be settled by other modes of experimentation.

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IMMUNITY IN PNEUMOCOCCAL INFECTIONS.

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Immunity in Pneumococcal Infections.

CONGENITAL immunity or unsusceptibility towards pneumococcal infection is possessed in different degrees by animals of different species, pigeons and domestic fowls being highly immune, the human species, guinea-pigs, rats, dogs and sheep being more susceptible, while rabbits and mice fall an easy prey to pneumococcal disease. Acquired immunity is that attained naturally, by passing through an attack of pneumococcal disease, or artificially, by the inoculation of the microbe itself or its products—active immunisation—or of the antibodies produced in the actively immunised organism — passive immunisation. But on the quantity and quality of the immunity, and on the mechanism by which immunity is conferred, unanimity of opinion has not been attained, though investigated from many directions and by widely varying methods.

Acquired Immunity.—The immunity naturally acquired by passing through an attack of the chief pneumococcal infection—pneumonia—does not appear to be very durable. Thus Rieselle (1) in 100 cases of pneumonia found that only 50 had it for the first time, that 32 had it for the second time, and the rest for the third time or oftener. Möllmann (2) in 832 cases noted that only 13 per cent. were in their first attack of pneumonia. In 1800 cases collected from the literature on this subject 420 had previous attacks of pneumonia (*i.e.*, 23·3 per cent.). The frequent occurrence, however, of empyæma, meningitis, arthritis, endocarditis, etc., following pneumonia, would suggest that no immediate immunity is conferred. Yet, in the majority of cases, individuals who have passed through an attack of pneumonia enjoy immunity for a short time at least, and animals which have been inoculated with pneumococcus cultures, and which have recovered, are more or less resistant for some time.

Active Immunity.—The methods employed in actively immunising animals have been very numerous, attenuated cultures, small doses of virulent cultures, filtrates of fluids containing the pneumococcus, cultures killed by heat or chemicals, etc. Many investigators, disappointed with the varying results obtained by these methods, endeavoured to concentrate the immunising substance. Thus, the Klemperer ⁽³⁾ brothers produced from filtered cultures a yellowish-white powder, which they regarded as the specific poison of the pneumococcus—the pneumotoxin—and they tried to obtain immunity by inoculating it on animals. Foa and Scabia ⁽⁴⁾ produced by similar means a “pneumo-protein.”

Passive Immunity.—In 1891, researches on the subject of passive immunity were published almost simultaneously by Foa and Carbone, Emmerich and Fawitzky, and the Klemperers. Emmerich and Fawitzky ⁽⁵⁾ demonstrated that by injection of the blood serum or of pieces of muscles or organs of immunised rabbits, animals could be protected against a dose of culture injected simultaneously or a short time before. Foa and Carbone were pioneers in the therapeutic employment of serum in pneumonia in man, and in the four cases treated the favourable issue was hastened, the critical fall of temperature occurring in 24 to 48 hours after injection. Serum from pneumonia patients, in different stages of the disease, was inoculated into animals, but with no beneficial result—in fact, in several cases the fatal issue seemed hastened; if used 24 hours after the infection no beneficial result was obtained in any case.

G. and F. Klemperer used therapeutically the serum taken from pneumonic patients by leeches or blisters in the treatment of artificial pneumococcal infection in rabbits, and with apparently favourable results. In the 12 cases treated thus, the crisis occurred immediately in 5, and there was a transient fall of temperature in 7.

Washbourn ⁽⁶⁾, however, noticed that the serum of immunised animals often completely failed to protect other animals from infection, and, to attain still higher degrees of immunity, injected a pony with broth cultures killed by heat, then agar cultures, and subsequently living and highly virulent broth cultures. Better results were obtained, the serum protecting animals against infection, but, on its employment in two cases in man, whether the cure obtained was due to the serum or not, Washbourn was uncertain.

The Italian investigator, Pane ⁽⁷⁾, immunised cows and donkeys.

For this purpose, he employed pneumococci of the highest virulence—such that one-twenty-millionth part of a cubic centimetre of a culture killed rabbits of any weight in six days. The asses' serum gave the best results, for an injection of 0.75 cc. into the auricular vein of a rabbit protected it against twenty times the minimal lethal dose, even when this was injected thirty to sixty minutes before infection; while, from the serum of a rabbit, 1 cc. was required to attain the same result. Quantities of 3 cc. of the asses' serum protected against 20,000 times the minimal lethal dose of pneumococcus culture.

Pane then applied himself to the treatment of pneumonia in man with his highly active anti-serum, but the results did not justify his expectations. He treated 32 cases, with three fatal issues, and is convinced that, in cases which are not severe, a fatal issue may be averted by doses of 20 cc. given early and frequently. The behaviour of Pane's serum with different races of pneumococcus was tested by Eyre and Washbourn⁽⁸⁾, and they found that it was active for four strains out of five, and was a valuable means of separating different strains.

The serum most widely used at the present day is that of Römer⁽⁹⁾. What he attempts to do is to produce a highly bactericidal anti-pneumococcic serum, starting with the assumption that the antibodies produced in the serum were of the nature of amboceptors. The specificity of the immune bodies may be so great that in infection by one strain of a particular bacterium, the amboceptors formed are not capable of attaching themselves to the bodies of the bacteria of another strain, and, therefore, to have a universally active anti-serum, amboceptors suitable to all variations of the bacterial species pathogenic to man must be inoculated. Since the labile complement cannot be preserved, we can have only the amboceptors to deal with in a bactericidal immune serum. There are great difficulties connected with the production of fresh complements, because the inoculation of fresh serum may give rise to the production of anti-complements in the organism (Ehrlich and Morgenroth). To overcome these difficulties, Römer employs the mixture of immune sera from various animals (horse, bovines and sheep); each of these animals has been immunised against different strains of pneumococcus (according to the principle Tavel employs in the production of antistreptococcic serum) and mixes them. Thus Römer's serum is polyvalent in a double sense, in that it contains different immune

bodies suitable to the most varying complements, and, at the same time, suitable to being anchored to a great variety of strains of pneumococcus. The practical value of this serum has been favourably extolled by Römer, Axenfeld, and Krückmann, in the treatment and prophylaxis of *ulcus serpens corneæ*, but of its use in acute pneumonia the results have not been of such signal success. Thus, Pässler (¹⁰), in 24 cases, had a mortality of 4 (16·6%); he does not advise the general use of the serum, but affirms that, in certain cases of danger, it may be of some service, as the general condition seems to improve after each injection.

Of the value of Pane's serum the most conflicting results have been published. Fanoni (¹¹) regards Pane's serum as absolutely specific, and Dasaro-Cao (¹²) says that the serum acts promptly as a cure, and the mortality has fallen one-third. Banti and Pieraccini (¹³) came to the conclusion that Pane's serum gave no beneficial result in any of their cases, even in large doses. Reports on the value of anti-serum have been given by de Renzi (¹⁴), Concetti (¹⁵), Righi (¹⁶), Spolverini (¹⁷), and other Italians; by Goldsborough (¹⁸), Sears (¹⁹), Snively (²⁰), Pässler, etc. etc. The largest number of cases collected from the literature is that of Goldsborough, who published a record of 447 cases with a mortality of 16·5 per cent. Up to the present time (end of 1905) 515 cases have been recorded with a mortality of 15 per cent., which does not show a great deal of improvement on the 17·9 per cent., the average mortality in untreated cases of pneumonia (Fussell).

Treatment was also tried by use of the serum of pneumonic convalescents by the Klemperers, Spolverini, Neisser (²¹), Audeoud (²²), etc., but with most indifferent results.

Mechanism of Immunity.—As to the mechanism by which immunity has been acquired, different opinions have been entertained by the different investigators. Thus the Klemperers, thinking that an antitoxin (antipneumotoxin) was produced in the blood as in diphtheria and tetanus, regarded the action as not directed against the killing of the cocci, but against the neutralisation of the toxin. A substantiation of their opinion they found in the fact that a filtered bacterial-free broth-culture when mixed with immune serum caused no rise in temperature, or only a temporary rise, but alone it killed an animal with the manifestations of high fever. They ascribed the cure of pneumonia in man to an assumed antipneumotoxin production in the blood of the

patient, in such quantity as to neutralise the toxin completely. The organism could then quite easily destroy the toxin-free cocci, and when this was complete the crisis took place. If one supplies to the serum, therefore, the serum of immunised animals containing much antitoxin, the individual will be freed from pneumotoxin much more rapidly; hence the curative action of the immune serum.

Mosny was of the opinion, ascribing to the immune serum a "toxicidal" action, since the diplococcus in this serum preserved its vitality and virulence for a month, while it was destroyed in non-immunised animals in four days. In connection with this, Tizzoni and Panichi (²³) have recently showed that it sometimes required several months for the pneumococci to be destroyed by the serum even of highly immunised rabbits.

Bonome (²⁴), however, attributes the immunity acquired during an attack of acute pneumonia to the bactericidal property of the blood, and Emmerich voices the same opinion, though he supposed that in the blood of immunised animals there is a substance, arising from the union of the globulin with the toxin shed, or with the toxin in the bacterial cell itself. This substance, which he calls the "Immuntoxinproteid," has great difficulty in acting on the tissue cells, and is therefore innocuous for those, but it can easily penetrate the bacterial cell, where it is split up into toxin and immunoprotein, which, being both in the nascent state, act as virulent poisons for the cocci and rapidly destroy them.

The French school of workers, headed by Metchnikoff, ascribe the immunisation against pneumococcus entirely to phagocytosis. Issaëff (²⁵), after a series of most careful experiments, is convinced that there is no bactericidal and no antitoxic power in the serum or tissue-juices, and he ascribes the whole rôle to the leucocyte. Pane is also an active adherent of this theory, and explains the action of his serum on the supposition that, by the introduction of the immune serum, the leucocytes are excited to secrete materials which protect animals against the pneumococci.

In a series of extensive researches, published by Tchistovitch, of St. Petersburg (²⁶), the exact conditions in the lungs of pneumonic patients and of dogs, in which pneumonia had been artificially induced, were carefully studied. In imitation of Patella's work (1888), he made

lung punctures with a Pravaz's syringe before, during, and after the crisis, and inoculated the exudate thus obtained into rabbits and mice. He thus was able to demonstrate that, even after the crisis, one could obtain from the lung an exudate containing not only viable, but even virulent, diplococci, killing the animals rapidly by septicæmia. Thus the crisis does not depend on the killing of all the diplococci in the lungs, for they may be found in considerable abundance, yet on culture the number of organisms was shown to be comparatively few.

Pneumonia was then induced in dogs by intra-tracheal injection of pneumococcus culture, which was allowed to flow down by gravity into the lower pulmonary lobes. Graduated doses were used, and in several cases the most intense pneumonia was set up. The animals were killed at different intervals, the lungs hardened, sections cut, and stained by the Gram-Weigert method. Thus he was able to demonstrate—

1. That in the cases going rapidly to a lethal issue, the phenomena of phagocytosis by the leucocytes lying in the alveoli were entirely absent.

2. That in cases going on to cure, but killed during the course, there was a large exudate of phagocytes in the alveoli, with numerous cocci lying within them.

By this method he was also able to show that, in non-resistant animals, the diplococcus causes only a very feeble local inflammatory reaction, and little phagocytosis is present; the leucocytes do not engulf the cocci, which can therefore go on rapidly multiplying, and quickly cause a fatal issue. In resistant animals, the inoculation of pneumococcus causes an inflammatory process of varying intensity, with dense cellular infiltration and a more or less pronounced phagocytosis. In a further series on animals inoculated with a weak pneumonic virus, Tchistovitch showed the close parallelism between the course of the disease and the leucocytosis, and considered the crisis as mostly brought about by phagocytosis, the intoxication ceasing with the taking up of the cocci. The protective bodies occurring in the serum he regarded as adjuvant factors, the hypothetical stimulins stimulating active englobation by the leucocyte, and the agglutinins causing the massing together of the cocci, and thus rendering their rapid ingestion by the phagocytes more easy.

But it was destined that a severe blow should be inflicted on the pre-eminence of the leucocyte. The first indication of this was the work of Denys and Leclef (²⁷) on the mechanism of streptococcal

immunity, in which they demonstrated that, besides the bactericidal action of the serum, to which they faintly adhered, there was a more important factor at work—phagocytosis brought about under the influence of the serum. “*Chez le lapin vacciné, le leucocyte tient du serum son pouvoir d'englober et détruire le streptocoque.*” A very short time later Mennes (²⁸), working under the direction of Denys, extended these researches to the pneumococcus, and was able to show that the primary immunising element is in the serum and not in the leucocyte. Highly immune sera were taken and carefully tested for their bactericidal and bacteriolytic power, and in no case was either of these apparent, even after adding quantities of normal serum as a complement. But when leucocytes were added to such sera, the cocci were rapidly picked up by them and underwent a gradual degeneration and lysis within them. In many cases the cells were packed full of bacteria, whereas with normal sera the number of bacteria in the phagocyte was comparatively small.

The chief question then arose—Has the serum, as Metchnikoff and his school believe—a mere stimulating action on the cells, or does it act directly on the bacteria themselves?

In 1903-1904, Wright and Douglas (²⁹) found that the phagocytic property of leucocytes suspended in a serum which has been subjected to a temperature of 60-65° C. is entirely in abeyance, and, on the addition of bacterial emulsions, no phagocytosis takes place. If, conversely, the unheated serum is mixed with bacteria, and digested for fifteen minutes at 37° C., and then subjected to 60° C. for a quarter of an hour, phagocytosis takes place on adding leucocytes. From these fundamental facts it is manifest that some definite substance has passed out of the serum, and attached itself to the bacteria, rendering them suitable pabulum for phagocytosis by the leucocytes. This thermolabile substance they named “Opsonin.”

During the process of active immunisation with staphylococcus, micrococcus melitensis, bacillus tuberculosis, and bacillus pestis, they have been able to show that for these infections the opsonic content of the serum is increased, and have thus demonstrated that, in addition to anti-toxic and bactericidal immunity, there is a third type—opsonic immunity, which is a common phenomenon in a number of infections, and brought about by a coalition and inter-action of the body humors and cells.

Recently, in examination into the phenomena of the immunity acquired in passing through an attack of croupous pneumonia, I made an investigation into the course of the opsonic content of the serum during this disease and in artificial infections in animals. Fifty-five cases were at my disposal, twenty-five of these being more or less typical cases of acute croupous pneumonia; nine others were in children with the clinical diagnosis of acute croupous pneumonia, seventeen cases were definitely broncho-pneumonia, and the remaining four were cases of post-pneumonic empyæma from which the pneumococcus was isolated in more or less pure culture.

The technique employed was that of Wright and Douglas. Three parts of the serum of the patient were added to three parts of my own citrated and washed corpuscles and an emulsion of pneumococci. These were thoroughly mixed and incubated at body temperature for fifteen minutes. Films were then made of the contents of the mixing pipette, dried and stained by Leishman's dye. The number of pneumococci in thirty to fifty polymorphonuclear leucocytes were counted and an average per leucocyte determined. Simultaneously with each experiment a control, or series of controls, was made with the sera of normal healthy individuals. The result is expressed in terms of the "opsonic index," the average number of cocci per polymorphonuclear leucocyte when the serum added is that of a normal individual being regarded as unity, and the opsonic index of the pneumonic patient is expressed in relation to the normal opsonic index of a healthy person.

The strains of pneumococci used in these experiments were seven in number, being isolated from empyæmata and other pneumococcal conditions, and many of them were employed a few days later in testing the sera of the patient from whom they were isolated. All the strains reacted similarly to variations in the opsonic index. The great essential in the preparation of the pneumococcal emulsions is the securing of homogeneity. One strain of pneumococcus had to be discarded owing to the cocci adhering together in clumps, and was therefore quite unsuitable for the determination of the average number of cocci in a number of leucocytes. The best means of attaining uniformity is to thoroughly shake up the emulsion and then centrifugalise for a few minutes. The masses and clumps are then deposited and the uniform upper layer of emulsion employed in the tests. The cultures were twenty-four hours

old; in no case were cultures older than forty-eight hours used. The culture medium employed was glycerine-agar.

Of the twenty-five cases of acute croupous pneumonia, fifteen were examined regularly from day to day throughout the course of the disease, and the opsonic index of the serum determined before, during, and after the crisis. In the other ten cases it was only possible to obtain the serum at less frequent intervals, but the results in those in no case disagreed with the opsonic curve determined for the others.

It will be convenient to discuss the relation of the opsonic index to :

(1) The chill period.

(2) The course of the disease.

(3) The final result, whether going on to rapid or tardy resolution, to a fatal issue, or to complications.

(1) At the chill period I have only been able to examine the serum of one patient. This was obtained two hours after the initial rigor, while the patient was feeling very cold and shivery. The opsonic index stood at '65; the original index was unknown, but in a healthy girl, such as the patient, was probably near that of a normal person.

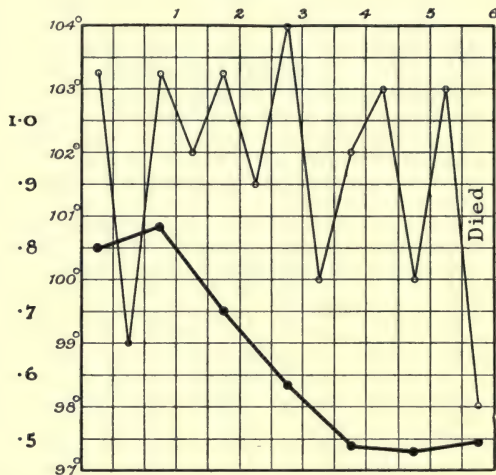
(2) During the evolution of the disease the opsonic index of the disease is considerably below unity, affording indices varying from 0'45 to 0'8. The lowest index observed was 0'45, in a poor under-nourished woman, who rapidly succumbed. In fact, all the cases in which the opsonic index fell below 0'5 terminated fatally. Considerable daily variations are present in the development of the disease. A progressive fall in the opsonic content of the serum does not seem invariably to indicate a fatal issue, though such a fall was observed in most of the fatal cases (see Chart I.).

(3) On the incidence of the crisis the greatest changes take place in the opsonic index. For some twelve hours the amount of opsonin in the serum is vastly increased, and with the increase the patient's condition seems to improve very greatly, and the critical phenomena occur. The amount of opsonin began to increase before any fall in the temperature was noticeable in many cases in which, with a high temperature in the morning, but a normal or higher opsonic index, one could almost predict the critical fall to take place during that day. A pseudo-crisis is not associated with increase in the opsonic index.

In cases undergoing rapid resolution the opsonic index rises suddenly to above normal, reaching 1.1 to 1.6. When the resolution is more tardy in nature, the rise is, as a rule, not so high, but a slight increase to the normal comes on and persists, the temperature and the general condition improving gradually a day or two later (Charts II. and III.). In the cases of rapid resolution, the index again falls to normal in periods varying from one to three days.

In fatal cases the opsonic index goes on diminishing till the *exitus lethalis*. This was constant in the five cases with fatal issue. In one case the temperature fell to normal, after a typical pneumonia, but the opsonic index did not rise. He died on the following day.

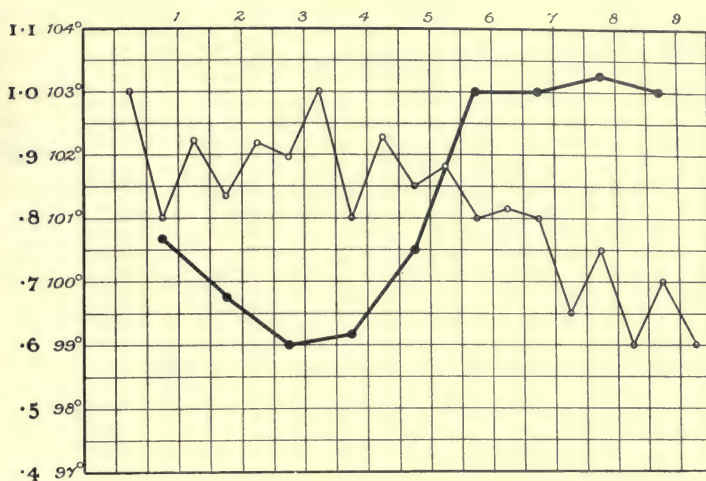
CHART I.



A fatal case of pneumonia. The thicker line and numbers represent the opsonic curve; the other, the temperature.

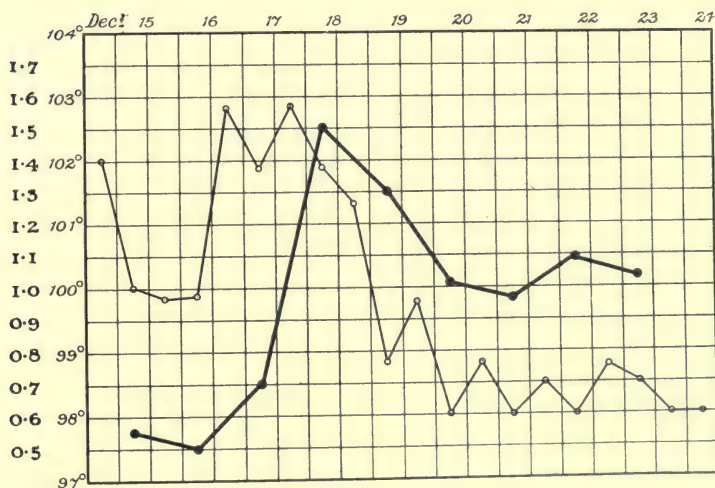
Of complicated pneumonia, I have examined two cases of empyæma, one of meningitis, one in which the opposite lung was attacked after the first crisis. The meningitis case died in three days with falling opsonic index. (The pneumococcus was isolated from the cerebral membranes *post mortem*). One of the empyæma cases died, the other survived. In the fatal case the pneumonia seemed to crisis, but not satisfactorily, for, two days later the opsonic index rose, and continued rising; the

CHART II.



From a case of pneumonia undergoing gradual resolution, and showing the gradual rise of the opsonin index (indicated by the thicker curve) to unity.

CHART III.



From a case of pneumonia undergoing rapid resolution. The thicker line and numbers represent the opsonic curve, the others that of the temperature. The opsonic index rises above the normal, and falls to normal two days after the crisis.

pleural cavity was then aspirated and a large quantity of pus containing pneumococcus withdrawn. In spite of this, the patient died with an index above normal. In the other case the opsonic index rose to 1·5, but on opening the pleural cavity it fell to normal and healed quickly.

The amount of opsonin present in the serum and exudate in post-pneumonic empyæma was examined in four cases. Case 1 gave an opsonic index of 1·4. Twenty-two ounces of pus were aspirated from his chest (pure culture of pneumococcus). The opsonic index of the liquor puris compared with the blood serum was 0, the leucocytes refusing to pick up a single coccus. Two days later the empyæma was drained; the blood-serum at time of operation had opsonic index of 1·35, and the liquor puris of the exudate 0·5. Next day, that of the serum was 1·37, and of the exudate 1·36. The progress of the case was rapid, the opsonic index of the serum and fluid of the exudate were tested successively for ten days, and both adjusted themselves gradually till both were approximately normal. The exudate always gave a slightly lower reading than the blood-serum. The blood-serum of Case 2 gave an opsonic index of 1·1 on the day subsequent to draining operation on his pleural cavity, while the exudate gave an index of 0·99. The infection was a pure pneumococcus one, and the discharge ceased within a week. Case 3 was a child, and fatal. Along with pneumococcus, *Staphylococcus albus* was present. Two days before death the opsonic index of the serum was 1·2, and that of the exudate 0·3. Case 4 had a discharging empyæma, subsequent to pneumonia, for two years. The organisms isolated were *Bacillus coli*, *Staphylococcus albus*, etc. The opsonic index of the serum of the blood was 0·55, and that of the discharge 0·52. Treatment by continuous negative pressure was continued for two months, during which he improved greatly. At the end of the time the opsonic indices in the blood-serum and exudate fluid were 1·08 and 0·85 respectively.

The seventeen cases definitely diagnosed as broncho-pneumonia came from the children's wards. These were similarly tested with pneumococcus and their indices determined. The results in these cases are very much less definite than in genuine croupous pneumonia in adults. In some of the cases with a high temperature and signs of a severe infection the opsonic index was normal throughout. In most of the cases the results are so varying that a definite curve for the course of opsonin in this affection could not be attempted. Of the nine cases in children diagnosed

clinically as acute croupous pneumonia, three gave charts very similar to those of adults; the others were more or less irregular.

The significance of the variations in the opsonic content in the serum in acute pneumonia.—In the majority of patients suffering from staphylococcic infections, as furunculosis, sycosis, acne, etc., or tuberculous infections, as phthisis, lupus, Addison's disease, etc., the opsonising power of the serum has been found to be below that of a normal individual. The same is true of pneumococcal infections and in the pre-critical period of acute pneumonia. The critical changes in the course of pneumonia must be regarded as phenomena of immunisation. From the nature of the opsonin it is manifest that, with the rise in the amount of opsonin in the serum, and accordingly in the serum bathing the leucocytic exudate in the pulmonary alveoli and capillaries, more pneumococci are prepared for the leucocytic meal, and they are taken up in greater number, the intoxication of the individual rapidly ceases, and the disordered metabolism is swiftly restored.

Should, however, the amount of opsonin in the serum fall instead of rise, the quickly proliferating cocci are not taken up in such numbers by the phagocytes; the pulmonary alveoli and capillaries thus contain many free cocci, a condition of bacteriæmia results, and with an aggravation of this condition intense intoxication and death.

In the case of empyæma developing from pneumococcal pleuritis, the numerous cocci present and proliferating between the layers of pleura absorb all the opsonin from the exudate effused. On opening such a pleural cavity fresh serum laden with opsonin is poured out into the cavity, the cocci are taken up rapidly by the leucocytes in the granulating zone and exudate and destroyed. The condition, therefore, tends to go on to healing should the opsonic content of the serum be sufficiently high. If it be low, it is manifest that the serum poured into the opened pleural cavity would also be low, the cocci would be less efficiently picked up by the leucocytes, and the disease would tend to continue for a longer period and become an essentially chronic discharging empyæma.

Analogous cases are tuberculous and staphylococcic abscesses, in which, as Wright and Bulloch have demonstrated, the fluid of the pus of a closed abscess possesses no opsonic power, although the blood of the patient exhibits it in a considerable measure. If these be opened, the lymph afterwards flowing from the wound has high opsonic power.

The high opsonic index registered in cases of closed empyæmata may be regarded as an expression of immunisation from resorption of immunising products from the pleural cavity. When opened, and the conditions favouring resorption removed, the opsonin in the serum is restored to normal in a shorter or longer period.

That the variation in opsonin in acute croupous pneumonia is a specific variation for the pneumococcus is shown by testing the opsonic content of the blood for another bacterium—*e.g.*, the staphylococcus. Thus it is shown that while the pneumococcal opsonin undergoes great changes in quantity during the course of acute croupous pneumonia, the opsonic content for staphylococcus varies from the normal in only a slight extent. Several of the cases were tested throughout, and in each case this was verified. Thus, one day three patients gave indices for pneumococcus of 0·5, 0·65, and 0·95, and staphylococcus 1·09, 0·85, and 1·0 respectively. Two days later, the same cases were tested for both organisms, giving indices of 0·85, 1·3, and 1·03 respectively, while the staphylococcal indices were 1·0, 0·86, and 1·01 respectively.

Another method of demonstrating the specificity of opsonin in the serum is by an absorption experiment. Serum is taken and the mixture digested with a measured quantity of thick pneumococcal emulsion in normal salt solution for fifteen minutes at 37° C. A similar measured quantity of normal saline solution is added to the control. The cocci are then removed by centrifugalisation and the supernatant fluid tested against the control. The result is: Control for pneumococcus, 1·00; supernatant for pneumococcus, 0·12; control for staphylococcus, 1·00; supernatant fluid for staphylococcus, 0·95. The remainder of the supernatant fluid is divided into two equal parts, and a quantity of thick staphylococcus emulsion added to one while an equal quantity of saline is added to the other half. Both are digested for fifteen minutes at 37° C. The cocci are deposited by the centrifuge. The supernatant fluid is tested with pneumococcus, staphylococcus, and tubercle bacillus.

	For Pn.	For Staph.	For T. B.
Opsonic content of control . . .	1	1	1
" " supernatant fluid	0·10	0·13	0·76

From these experiments it may be deduced that there is an opsonin specific for each organism or group of organisms (the opsonin for *Staphylococcus aureus* does not seem to be different from that of *Staphylo-*

coccus albus), and the changes in the opsonic content in a particular infection concern only the opsonic content with reference to the infecting agent.

In the course of active immunisation of animals against the pneumococcus, the opsonic content of the serum follows a fairly definite course.

On inoculation of a rabbit with 1,000 millions of pneumococci killed by heat (counted by Wright's method), the opsonic index fell to .7 and .65 on the two succeeding days; on the third day, it rose to 1.3, and remained above normal for ten days.

A second rabbit was inoculated with 30,000 millions of pneumococci. The negative phase persisted for seven days, after which a very marked positive phase, giving indices of 1.6, 1.8, 1.9, followed, the serum not giving the normal index for four weeks after inoculation.

Many similar experiments with varying dosage gave results which would point to the conclusion that the smaller the dose the less pronounced and the less durable was the negative phase. The amount and persistence of the positive phase was not so constant, as in many cases, after a long negative phase from a large dose, the positive phase was entirely absent, the opsonic index gradually rising to unity.

The specificity of the opsonin was also apparent in these experiments on animals, for in some cases the serum was tested throughout against both pneumococcus and staphylococcus, and showed that while the opsonic index for pneumococcus was passing through wide variations, that for staphylococcus remained almost unchanged from day to day.

In the lower animals there are great differences in opsonic index observed between different species, but the individuals of any given species do not appear to vary much. For pneumococcus the indices of the laboratory animals are in comparison with man 0.4, 0.6, and 0.8 for rabbits, guinea-pigs, and rats respectively. On the other hand, their opsonic index for the tubercle bacillus is found to be 0.6 and 0.4 for rabbits and guinea-pigs respectively. Their opsonic index seems to correspond very closely with their susceptibility to infection.

Nature of the Opsonic Substance in the Serum.—Before making any systematic enquiry into variations in the opsonic content of the serum, it is necessary to enquire into the effects of preservation, effects of light, of dilution, of addition of salts, etc.

Effect of preserving the Serum.—Samples of blood were drawn from

the finger each day for 12 days, and kept in the dark in unsealed capsules. The sera thus obtained were compared with fresh serum for opsonic content (in all the experiments my own washed blood corpuscles were used), and the experiments on normal serum were conducted on my own serum or that of healthy normal individuals.

				Cocci per leucocyte.
Fresh serum, 2 hours old	-	-	-	17'6
Serum, 24 hours old	-	-	-	16'8
" 48 "	-	-	-	15'4
" 3 days old	-	-	-	14'3
" 5 "	-	-	-	14'2
" 8 "	-	-	-	10'3
" 10 "	-	-	-	8'5
" 12 "	-	-	-	6'5
Serum over 3 weeks old	-	-	-	2'5

Thus it is seen that there is a gradual diminution from the first day onward, and that it would give quite erroneous results if we were to compare sera of different ages with regard to opsonic content.

What are the changes the serum undergoes within the first few hours of its withdrawal from the body?

Blood was drawn from the finger at different times during the day, and left exposed to daylight when all the samples were tested simultaneously.

Blood drawn at	Tested at	Exact age.	Cocci per leucocyte.
11'55	3'51	3 hrs. 56 mins.	13'7
12'55	3'49	2 " 44 "	13'1
1'25	3'47	2 " 22 "	12'9
1'55	3'44	1 " 49 "	13'1
2'10	3'38½	1 " 28½ "	11'3
2'25	3'36	1 " 11 "	10'4
2'40	3'29	49 mins.	9'9
2'55	3'27	32 "	9'7
3'10	3'25	15 "	9'0
3'30	3'42	12 "	9'5

Thus it is shown that there is a gradual increase up to about two hours in the quantity of opsonin in the serum. It may be asked, however, does the increase in opsonin depend on a change in the serum itself, or is it due to certain substances squeezed out of the clot?

The following experiment solves this question. A large quantity of blood was drawn, and the corpuscles driven down to the bottom of the capsule by centrifugalisation. Quantities of the serum were then drawn off at intervals, and stored in sealed glass pipettes. The contents of these pipettes were all tested simultaneously with normal corpuscles and bacterial emulsions.

Serum removed from clot after	5 mins.	-	-	-	6.7	
"	"	15	"	-	-	6.9
"	"	30	"	-	-	7.5
"	"	45	"	-	-	7.8
"	"	60	"	-	-	8.8
"	"	75	"	-	-	9.7
"	"	90	"	-	-	10.1
"	"	2 hrs.	-	-	-	10.7
"	"	2½ hrs.	-	-	-	10.3
"	"	3 hrs.	-	-	-	10.1
"	"	3½ hrs.	-	-	-	10.5
"	"	4 hrs.	-	-	-	10.2
"	"	4½ hrs.	-	-	-	10.7

Thus, contact with the clot seems to be necessary for increase in the opsonising power. This increase was observed in every instance, and it seems a constant law that the opsonic power of the serum increases up to a period of two hours, after which it gradually diminishes so that, by the seventh or eighth day, it has lost 50 per cent. of its opsonin.

A probable explanation of this property is that the clot acts as a permeable filter, which, as it contracts, squeezes out constituents in a definite order, according to the size of the molecule :

1. Sodium chloride and salts ;
2. Semicolloids ;
3. True colloids, to which there is much evidence that substances of the opsonin type belong. This hypothesis is substantiated by the fact that the serum squeezed out last from the contracting clot contains more opsonin per volume than that squeezed out at any earlier period in the contraction.

From these figures, it is evident that, before the sera of two individuals can be compared accurately with regard to their opsonic indices, they must be subject to the same physical conditions for similar periods.

Effect of Temperature.—If the serum of a normal healthy individual be exposed to a temperature of 55 to 60° C. it loses its opsonic power almost entirely; never, however, completely, for even after heating to 60° for one hour there is always a small irreducible minimum left.

Normal serum + Normal corpuscles + Cocci					Cocci per leucocyte.	
					18'2	
Normal serum, heated to 60° for	2 mins.	+	"	+	"	15'3
"	"	4	"	+	"	13'2
"	"	6	"	+	"	10'7
"	"	10	"	+	"	5'5
"	"	15	"	+	"	5'3
"	"	20	"	+	"	3'7
"	"	30	"	+	"	3'5
"	"	40	"	+	"	3'3
"	"	50	"	+	"	3'1
"	"	60	"	+	"	2'9

Heating to 60° for 60 minutes destroys 83 per cent. of the opsonin contained in the serum, and therefore the opsonin in the serum of normal individuals may be regarded as thermolabile.

That the irreducible minimum after heating normal serum is still opsonin is shown by an experiment in which a large quantity of cocci are passed through the heated serum and afterwards removed by the centrifuge.

A quantity of normal serum is heated for 30 minutes in the water-bath at 60° C. A thick emulsion of cocci is added, and the mixture allowed to digest for 15 minutes. The cocci are then removed by centrifugalising for two hours. Result:—

										Cocci per leucocyte.
Normal serum	-	-	-	-	-	-	-	-	-	15'3
Normal serum heated to 60° for 30 mins.	-	-	-	-	-	-	-	-	-	2'4
Serum after treatment as above	-	-	-	-	-	-	-	-	-	0'14

The substance has evidently combined with the cocci, and is thus also opsonin.

The rôle of the Leucocyte.—The comparative indifference of the behaviour of the leucocyte in phagocytic phenomena was shown by the early experiments of Wright, and later by Bulloch and Atkin (³⁰). In a comparison of normal serum and that of a lupus patient, Bulloch and

Atkin found that the leucocytes of a lupus patient and those of a normal individual were approximately identical in action. To quote their experiment :—

1.	Serum of a normal individual + T. B. Emulsion + leucocytes of a normal individual	-	-	5.7
2.	" " + " + leucocytes of a lupus patient	-	-	5.4
3.	" " + " + " "			5.3
4.	" " + " + " "			5.2
5.	Serum of a lupus patient + T. B. Emulsion + leucocytes of a normal individual	-	-	2.5
6.	" " + " + " "			2.4
7.	" " + " + " "			3.2

I have determined the behaviour of the leucocytes in acute croupous pneumonia, a disease in which there is pronounced changes in the number of leucocytes at the different periods.

Serum of Normal individual + Pneumococci + normal leucocytes	-	-	13.2
Serum of Pneumonia patient No. 1 + Pneumococci + normal leucocytes			6.5
Serum of Pneumonia patient No. 2 + " + "			9.7
Normal serum + Pneumococci + leucocytes of Pneumonia patient No. 1			9.5
Normal serum + " + " " No. 2			8.4

From these results it would appear, at first glance, that the leucocytes were not so indifferent, and that the leucocytes of a pneumonia patient were not capable of taking up so many cocci in the same period of time. But the number of leucocytes in the experiments was here variable, for, in the normal blood, on the day in which the experiment was performed, there were 8,200 leucocytes per cm.m., in pneumonia patient No. 1, 18,000, and in No. 2, 19,200. Many more leucocytes were present, and actively phagocytic, in the last two cases.

				Total in 50 leucocytes.
Normal serum + Normal leucocytes + Pneumococci	-	-	-	660
" + Leucocytes of Pneumonia patient No. 1 + Pneumococci				475
" + " " No. 2 + "				420

But if numbers in proportion to the number of polymorphonuclears of patients with pneumonia were taken, the results show that the poly-

nuclears of pneumonia patients are equally active to those of normal blood.

Total number of cocci in 40 leucocytes of normal blood	-	-	-	540
" " 90 " Pneumonia patient No. 1	-	-	-	700
" " 85 " " No. 2	-	-	-	720

In some of the cases it was very remarkable how wonderfully the counts in relative numbers totalled up. In a case of pneumonia in which there were, on the days of estimation, 12,500 leucocytes to normal 8,700, the results were :—

				Cocci in 50 leucocytes.
Normal serum + Normal leucocytes + Pneumococci	-	-	-	473
" + Pneumonia patients' leucocytes + Pneumococci	-	-	-	376

But the number of polymorphonuclears of normal blood to that of the patient's blood was approximately 1 : 1·4, and, if one adds two-fifths to the pneumonia patient's count, we have 470, as compared with 473 in the normal man.

Effect of dilution of the Serum.—Many experiments were performed on the effect of diluting the serum of the individual examined by normal saline solution (0·85 per cent.). Serum of a normal individual was diluted with definite quantities of saline solution. Three parts of the normal serum and three parts of each of the diluted specimens were added to the leucocytes of a normal individual and an emulsion of cocci. The mixtures were incubated at 37° C. for 15 minutes, films made, and the total number in 80 leucocytes estimated and an average determined. Result :—

					Cocci per leucocyte.
Normal serum 3 parts	-	-	-	-	10·9
Normal serum 2 pts. + Normal saline 1 pt.	-	-	-	-	10·7
Normal serum 1 pt. + " 2 pts.	-	-	-	-	10·2
Normal serum ½ pt. + " 2½ pts.	-	-	-	-	5·9
(Normal serum : Normal saline = 1 : 8) 3 pts.	-	-	-	-	2·0
(" " = 1 : 10) 3 pts.	-	-	-	-	1·9

The conclusion drawn at first glance from these figures is that the opsonic index is *not* an index of the amount of opsonin in the serum, seeing that the serum can be diluted to as much as one in three with

very little change in the opsonic index. The time given for the interaction of the fluids, bacteria and leucocytes, did not allow either the cocci to be thoroughly opsonised or the leucocytes to take up the cocci that were opsonised. If the interaction is allowed to go on for 45 minutes, the result is somewhat different :—

	Cocci per leucocyte.
Normal serum 3 pts. - - - - -	16·7
Normal serum 2 parts + Normal saline 1 pt. - -	11·7
" 1 part + " 2 pts. - -	5·9
" $\frac{1}{2}$ part + " $2\frac{1}{2}$ pts. -	3·4
(Normal serum : Saline = 1 : 8) 3 parts - - -	1·9
(" " = 1 : 10) 3 parts - - -	1·8

The numbers in this latter experiment fall almost exactly proportional to the dilution.

From this and the preceding experiment the conclusion to be drawn is that the most accurate means of determining the opsonic content and opsonic index of any serum is to allow either the serum, leucocytes and bacterial emulsion, to interact for at least 30 to 45 minutes, or to dilute the serum one part in 3 parts with normal saline, if the period of interaction allowed is to be 15 minutes.

Effect of dilution with Na Cl. solutions stronger than 0·85 per cent.

	Cocci per leucocyte.
Normal serum 3 volumes - - - - -	10·5
Normal serum 1 vol. + ·85 % Na Cl., 2 vols. - - -	4·3
" + 1·0 % " - - -	2·0
" + 1·5 % " - - -	1·7
" + 2 % " - - -	1·6
" + 5 % " - - -	1·3

(These were incubated with leucocytes and coccal emulsion for 30 minutes.) Increasing the concentration in salt seems to diminish the amount of opsonising power of the serum.

It is affirmed by Buchner that the destruction of alexine on heating to 55° C. can be paralysed by adding salt. Whether the same was true of opsonin was tested. Normal serum was diluted in the proportion of

two parts of serum and one part of different strengths of salt solution, and then subjected to 55° C. for varying periods.

				Cocci per leucocyte.
I.	Normal serum (2 vols.)	+ 0.85 % Na Cl. (1 vol.)	unheated	- - 17.9
	"	"	heated to 55° for 5 mins.	10.6
	"	"	" for 10 "	6.9
	"	"	" for 15 "	3.1
	"	"	" for 20 "	2.0
II.	Normal serum (2 vols.)	+ 1 % Na Cl. (1 vol.)	unheated	- - 12.5
	"	"	heated to 55° C. for 5 mins.	7.9
	"	"	" for 10 "	2.1
	"	"	" for 15 "	1.3
	"	"	" for 20 "	0.8
III.	Normal serum (2 vols.)	+ 1.5 % Na Cl. (1 vol.)	unheated	- - 11.3
	"	"	heated to 55° for 5 mins	2.1
	"	"	" for 10 "	1.2
	"	"	" for 15 "	0.9
	"	"	" for 20 "	0.8

In the experiments with 2 % and 5 % NaCl. the opsonin seemed to be entirely destroyed by heating for five minutes to 55° C.

Far from preserving the opsonin from destruction by heat, the increasing concentration of salt seems to favour its destruction very greatly.

The work of Wright and Douglas, corroborated also by Bulloch and Atkin, has shown that the opsonin disappears from the serum when the serum is digested with bacteria at 37° C. or at 0° C. We have seen that serum heated to 60° C. for some time loses the great part of the opsonin present. Is this really a destruction of the opsonin, or does it pass into a modification (opsonoid), in which it does not fully prepare the bacteria for phagocytosis? Can a complement-like body be isolated as in hæmolysis at 0° C. which would give a reaction with heated serum?

An emulsion of staphylococci (pneumococci gave a similar result) was added to normal serum and digested for 30 minutes at 0° C. The centrifuge was then applied, and a supernatant clear fluid obtained with a deposit of staphylococci. Serum was also heated to 60° C. for 30 minutes.

						Cocci per leucocyte.
Normal serum	+ Normal leucocytes + Normal cocci -					19'1
Normal serum heated to 60° for 30 mins.	+	"	+	"	-	3'2
Supernatant fluid 1 pt.	} +	"	+	"	-	2'9
Heated serum 2 pts.						
Supernatant 2 pts.	} +	"	+	"	-	3'2
Heated serum 1 pt.						
Supernatant fluid 3 pts.	+	"	+	"	-	2'2

The deposit was then made into an emulsion with normal saline, and tested with normal leucocytes, which showed that the cocci were opsonised, and, therefore, that the opsonin had passed from the serum into the cocci. In hæmolytic experiments, if a similar procedure is undertaken at 0° C., there can be separated from the supernatant fluid a complement which would give a reaction with heated serum. This is evidently not the case with opsonin. It has been alleged by Crofton ⁽³¹⁾ that there are two bodies in normal serum contributing to opsonisation of bacteria, one thermolabile corresponding to the complement, and the other thermostable corresponding to the immune body. His experiments do not give much support to this deduction.

When bacteria are opsonised, heating for prolonged periods to 60° C. is unable to destroy their power of being taken by the polymorphonuclear leucocytes without the intervention of serum. When once opsonised it is impossible to opsonise further, and opsonised cocci do not appear to be capable of removing opsonin from normal serum.

Cocci were opsonised by digestion at 37° C., and the mixture of serum and cocci was heated to 60° for 30 minutes. The cocci were centrifugalised down, and the deposit added to normal serum, and allowed to stand for 30 minutes. The centrifugi was again applied, and the supernatant clear fluid tested against normal serum.

Normal serum + Corpuscles + Cocci = 11'8
 Supernatant fluid + " + " = 11'2

Thus, passing opsonised cocci through the serum does not rob it of any of the opsonin contained.

Is the increased opsonic power developed during immunisation of the same nature as that of normal present in the serum?

In the investigation of this question, sera from several sources were examined, serum from pneumonia patients at the crisis with high opsonic index, serum from a patient suffering from acne vulgaris who had been treated with injection of staphylococcus vaccine, and sera of rabbits actively immunised against staphylococcus and against pneumococcus.

Sera from each of these sources gave the same reaction towards physical agents. They shall be designated "Immune sera."

The only great difference in the behaviour of immune sera and normal sera towards chemical and physical influences is in relation to the effect of heat. Heating to 60° destroys 80 to 90 per cent. of the opsonising power of normal serum. In immune sera heat does not exert such a great influence.

The serum of a patient who had received repeated injections of staphylococcus albus was tested. His opsonic power, with relation to a normal individual, was 1·8. This was subjected to heat for 30 minutes.

1. Control serum -	-	-	-	-	-	-	-	14·8
2. Patient's serum	-	-	-	-	-	-	-	23·7
3. No. 1 heated to 60° for 30 mins.	-	-	-	-	-	-	-	2·9
4. No. 2	"	-	-	-	-	-	-	6·9

From this experiment the opsonin does not seem to be destroyed to such an extent by heat. Is the residue in the serum of the nature of opsonin, or is it another substance?

If it is opsonin, then it would be able to opsonise fresh cocci passed through it. To demonstrate this, several experiments were performed of which a type may be cited.

Immune serum and normal serum were heated to 60° C. for 30 min., the thermolabile opsonin being thereby destroyed. To the heated sera were then added equal parts of an emulsion of staphylococcus, and the mixtures were digested at 37° C. for 30 minutes. At the end of this time the mixtures were centrifuged, the supernatant liquid and the cocci being separately tested with the following result :—

	Cocci per leucocyte.
1. Heated normal serum -	2·9
2. Heated immune serum -	6·9
3. Supernatant fluid after digestion with normal heated serum -	0·9
4. Supernatant fluid after digestion with immune heated serum	6·0
5. Cocci after digestion with normal heated serum -	2·3
6. Cocci after digestion with immune heated serum -	2·1

Similar experiments on immune sera from various sources gave a similar result, though the amount of thermostable element varied greatly in quantity. It is not proportional to the amount of opsonin present, or to the opsonic index.

The opsonic indices of two individuals may be the same, though one has been subjected to active immunisation. Both may be unity, yet, when subjected to heat, the difference between the two becomes apparent.

Example :—

	Cocci per leucocyte.
Immune serum - - - - -	9·8
Normal serum - - - - -	8·8
Heated immune serum - - - - -	3·2
Heated normal serum - - - - -	1·1
Supernatant fluid after digestion—immune - - -	3·4
" "—normal - - -	0·8
Normal corps. + deposit from "immune" digestion -	2·0
" + " "normal" digestion -	2·1

It would appear, therefore, that the serum of an individual which has been raised artificially to the normal standard differs considerably as regards thermolability from that of a normal individual.

With regard to the nature of the residual body after heating, it is present in the serum, not destroyed by heat, it does not attach itself to fresh bacteria added, and yet it causes phagocytosis. Most probably it acts as a stimulant to the leucocytic amoeboid movement—a true stimulin.

In the active immunisation of animals, this residual substance appears previous to the increase in opsonic index, and disappears for a short time before the opsonic index has again fallen to unity.

Relation of the Opsonin to the Leucocytosis.—In the examination of typical cases of acute croupous pneumonia, it would appear as if the leucocytosis curve and that of the opsonic content were inversely proportional, and this is so close that *prima facie* one might be led to trace phenomena of cause and effect, either that the rise in opsonic content caused the fall in the number of leucocytes or *vice versa*. The best method of determining whether any causal nexus exists between the two factors is by animal experiment. Thus, a rabbit was inoculated

with 1 cc. of a killed agar emulsion of pneumococci subcutaneously at 11 a.m. when the leucocyte count stood at 8,000 per c.mm. The result was :—

At 1 p.m.	-	-	-	-	-	8,800 per c.mm.
At 2'15 p.m.	-	-	-	-	-	5,600 "
At 3'15 p.m.	-	-	-	-	-	5,060 "
At 4'15 p.m.	-	-	-	-	-	7,800 "
At 5'15 p.m.	-	-	-	-	-	8,400 "
At 6 p.m.	-	-	-	-	-	9,800 "

The next morning, the leucocytosis was 8,600, and on subsequent days 6,800, 8,900, and 8,350. The quantity of opsonin in the serum during the first day when any leucocytosis present occurred (leucocytosis from bacterial inoculations usually attaining a maximum in about 12 hours if injected subcutaneously, and 8 hours if intravenously), there was only a slight fall in the opsonin (at 4'15 p.m.); on the following day, when the leucocytes were normal in number, the opsonic index was 0'7; then 0'5 the day after; two days later 1'1, and then on the second day after that 1'7. Thus, in the case of a single inoculation of a bacterial irritant, the leucocytosis does not run a similar inverse course to that of the opsonic content. Is there then any physiological nexus between the two?

The course of leucocytosis in acute croupous pneumonia may, like the opsonin course above described, be conveniently treated under the headings—

1. Chill period.
2. During the evolution of the disease.
3. Terminal leucocytosis.

Chill Period.—Löeper examined two cases in this stage, with the teeth chattering, and found the total number of leucocytes raised to 24,000 and 18,000. This fact would indicate that the chill does not indicate the beginning of the pneumonia, but most probably, as in the inoculation above described, there was a period of hyperleucocytosis, as was some time ago suspected by Werigo, who affirms that the leucocytes rush in virtue of chemotaxis to the focus of infection, the hæmopoietic organs being simultaneously excited to send out the reinforcements necessary. By this hypothesis, the hyperleucocytosis would, like the chill, interpret

the general reaction of the organism and stimulation of the defensive apparatus by the bacterial irritant.

During the Evolution of the Disease.—The usual number is about 18,000 to 24,000 per c.mm. The maximal leucocytosis is always about the first day or two, and it diminishes slightly afterwards.

Terminal Leucocytosis.—In cases undergoing rapid resolution there is usually a sudden rise in the number of leucocytes just on the eve of the temperature-fall, by about 2000 to 6000: on the day of the crisis the number is much reduced, and the day following is almost always nearly normal. In cases terminating by lysis, the rise in leucocytosis just before the crisis usually fails to occur. In complicated or fatal cases the leucocytosis may not fall to normal, but go on increasing until the fatal end. With very severe infections, however, or in patients with poor resistance, alcoholic patients, etc., acute pneumonia may run its course without any leucocytosis whatever.

On a single intravenous inoculation of pneumococci, there is a pronounced leucocytosis in five hours. In acute lobar pneumonia the researches of Prochaska ⁽³²⁾, Cole ⁽³³⁾, and Fränkel ⁽³⁴⁾, show that the pneumococcus can be very frequently isolated from the blood of patients suffering from typical acute croupous pneumonia, if sufficient quantity of the blood is removed, at least 10 cc. being required. It would seem as if there were discharges, slight and at frequent intervals, of pneumococci into the pulmonary capillaries and veins from the morbid lung, a condition of bacteriæmia excited with pronounced stimulation of the hæmopoietic organs and a high degree of leucocytosis. The leucocytosis is to be regarded as the cellular reaction product of the organism on infection, the anti-bodies produced in the serum being the humoral reaction products.

Agglutination Phenomena in Acute Croupous Pneumonia.—In this affection there are two separate agglutination phenomena witnessed—agglutination of the red blood corpuscles, and bacterial agglutination. The commoner and more uniform is the chromocytic agglutination, of which some mention may be made.

Wharton Jones ⁽³⁵⁾ stated that in the blood of inflammatory diseases “the rouleaux are formed more rapidly, and run into masses which have large spaces between them, and thus the buffy coat is produced by this close aggregation and more rapid subsidence therefrom resulting.” If

one adds a small quantity of the blood serum of a patient suffering from acute pneumonia to a small quantity of the corpuscles of a normal individual and examine in hanging-drop, the red blood corpuscles are clustered into large-meshed masses. Dilution with normal saline in sufficient quantity abolishes this reaction. A better result was obtained in the later cases of acute pneumonia, in which I used long sedimentation tubes used for Widal's macroscopic typhoid reaction. The dilutions borne were not very high. Many refuse to become agglutinated in 1:4 or 1:10. In the case of empyæma, which had been discharging for two years, the blood serum agglutinated my corpuscles in dilution 1 in 20, and the fluid from the pus 1 in 4. The agglutination usually began one or even two days before the crisis, and continued for a week after. The agglutination of the red corpuscles does not seem to stand in close relation to bacterial agglutination, as the latter was very slight, if at all present, in cases where the red cells were very markedly agglutinated. That the property lies in the serum is of easy demonstration, since the hæmagglutinin is thermolabile, being destroyed by 60° C. like the opsonin and also the hæmolysin. The addition of normal sera to the hæmagglutinating serum is, as a rule, not capable of inhibiting hæmagglutination.

Bacterial Agglutination.—In 1891, a phenomenon very close to agglutination was observed by Metschnikoff ⁽³⁶⁾, even before the publication of the papers of Gruber and Durham in 1896. He noted that in the serum of rabbits immunised against the pneumococcus the growth was in long coiling chains, while the same organisms in normal rabbits' serum have the usual diplococcic form. These facts were amply verified and more closely investigated by Mosny, Kruse and Pansini ⁽³⁷⁾, Washbourn, Bezançon and Griffon ⁽³⁸⁾, and others.

The agglutination of the pneumococcus in the sense of the Gruber-Widal reaction with the microbes in bouillon culture after addition of quantities of specific serum was first described by Neufeld, who comes to the conclusions :—

1. The occurrence of a strong agglutinating serum in immunised rabbits is entirely independent of the degree of immunity.
2. The agglutination phenomena are specific for the pneumococcus.
3. The agglutination of the pneumococcus shows many peculiarities, among them the great resistance of the bacteria against high degrees of heat.

In none of the rabbits immunised by pneumococcus did I obtain an agglutinating serum. The serum of several cases of pneumonia examined agglutinated the pneumococcus markedly, but in none were such high dilutions as 1 in 40, 1 in 50, etc., obtained. In many cases the addition of equal parts of a fine pneumococcal emulsion to serum, and incubation for $\frac{1}{2}$ -2 hours at 37° C., succeeded in demonstrating that agglutination had taken place. On examining this microscopically, the individual cocci seem to become swollen and run together into masses, thus verifying the truth of Gruber's assumption of the swelling and cohesion of the outer bacterial coats. The dilutions are best performed by Wright's sedimentation pipettes, in which they can be quickly and accurately performed and incubated at body-temperature.

If an agglutinating serum be diluted 1 to 4, the agglutination is not so marked as in the undiluted serum, and microscopically also the appearance differs; the deposit is not so granular. The cocci do not appear so much swollen, and are joined together into coiling chains — "the Fadenreaction." As shown by Neufeld, the agglutinin is thermostable, but is destroyed by 100° C. It can withstand 60° C. for more than one hour. On heating the agglutinated cocci (in the undiluted experiments) at 60° C. for an hour, the clumping and swelling of the bacteria disappeared.

As regards agglutination, in the Gruber-Widal sense of the term, the cases examined lead to the conclusions:—

1. That it is not present in every case of acute croupous pneumonia: it is very frequently absent altogether in cases which "crisis" before the fifth day, and the longer the time till the crisis occurs, the better is it marked.
2. That the serum is very insusceptible of dilution, 1:4 being considered as a high dilution.
3. That it is essentially a critical phenomenon, appearing at, and disappearing soon after, the crisis.

The most recent addition to our knowledge of immunity has its origin from the workers in the German University of Prague. Bail⁽³⁹⁾ has observed the great sensibility of tubercular guinea-pigs to fresh injections of living tubercle bacilli. On intraperitoneal injection, the animals die within a few hours, with an exudate in the pleural cavity, the cells

of which showed no phagocytosis whatever. Animals which had not attained this stage of hypersensibility either do not die at all, or only after some days or weeks, and then with well-marked phagocytosis in the exudate. He has shown that in the blood-serum of tuberculous animals or tuberculous exudate there is a substance which he names "Aggressin," which has the power of holding active phagocytosis in abeyance in animals in which the tubercle bacillus is a true parasite; and he has been able by successive inoculations of these exudates to attain high degrees of immunity. Hoke (⁴⁰) has applied himself to the investigation of the "aggressive" action of diplococcic exudates, and comes to very remarkable and important conclusions. The rabbit is employed, being an animal in which the pneumococcus is a true parasite. By intrapleural injection of cultures of pneumococcus isolated from a lung abscess secondary to acute croupous pneumonia, a rabbit was killed in 8 to 10 hours, and, *post mortem*, 20 cc. of exudate was obtained from the pleural cavity. In this exudate numerous cocci were found, but no phagocytosis. The exudate was centrifugalised for 12 hours or longer, and all the cocci removed and subsequently carefully sterilised with toluol. By prolonged standing in the incubator the toluol separates and forms a distinct layer, which can easily be pipetted off the top of the serum.

Should such a pneumococcal exudate possess "aggressive" properties, it must be capable of shortening the duration of the disease, because it holds in abeyance the natural protective power of the body, and allows the bacteria to multiply rapidly, without being engulfed by the phagocytes. Intrapleurally and intravenously were injected in one series of rabbits fresh pneumococcus emulsions in quantities of 0.2 to 0.5 cc., along with a quantity (2 cc.) of exudate: into the control series, instead of the exudate, normal saline was injected. The first series died in every case earlier than the control.

A remarkable property of "aggressive" exudates is that the aggressin action can be inhibited by the addition of leucocytes. This was also shown by Bail (³⁹) for cholera, and by Hoke (⁴⁰) for staphylococcic exudates, though first demonstrated by Kikuchi (⁴¹). Aleuronat was injected into the pleural cavity twelve hours before an injection of aggressin, and pneumococcal emulsion, and in that case death was much later than in the controls to which no aleuronat had been administered.

By treating animals with repeated injections of aggressin, great protective power is produced. An anti-aggressin is developed, by which phagocytosis is enormously stimulated. Thus, after three inoculations of an actively "aggressive" pleural exudate at intervals of five days, a rabbit remained alive with a dose of pneumococcus emulsion which killed another in 18 hours. Passive immunisation with the serum of rabbits treated with aggressin seemed also to protect against pneumococcal infection.

In the mechanism by which the different anti-bodies act in the production of immunity against pneumococcus, it will be seen that the great immunity agent is phagocytosis, in which the leucocyte, in itself a passive factor, is made capable of rendering great service to the individual by engulfing the pneumococci which have been massed into suitable clumps by the agglutinin, and rendered suitable pabulum by the opsonin, while the leucocyte itself is excited to labour by the stimulin in the serum. Aggressin also seems to be at the same time capable of exciting or paralysing phagocytosis, according to whether inoculated before or after infection.

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PRELIMINARY NOTE UPON THE BACTERIOLOGY
OF SOME DISEASES OF SHEEP.

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Preliminary Note upon the Bacteriology of some Diseases of Sheep.

THE heavy losses suffered by farmers on the west coast of Scotland from the prevalence of certain endemic diseases amongst sheep, led to the appointment by the Board of Agriculture of a Departmental Committee to investigate the aetiology of these diseases. Our investigation is to be regarded as an extension of the work of this Committee and of Professor Hamilton's own researches. (Parliamentary Report of the Departmental Committee, appointed by the Board of Agriculture, 1906.)

The material for this investigation was provided by Professor Hamilton, to whom we are indebted for suggesting the line of the research, as also for the very great help he has given us in the carrying out of the same. Liquid from the peritoneal cavity of sheep which had died from the various diseases had been preserved, and it was from this that the various organisms were isolated and grown. The method of isolation, in almost every case, was as follows :—The medium employed was glucose-bouillon of varying degrees of alkalinity. The surface was covered with olive oil to a depth of one centimetre. The oil allowed the escape of the gases dissolved in the medium during sterilization, and prevented later any further access of air (see Parliamentary Report, Part II., p. 20).

A tube of bouillon heated to 80° C. was inoculated with a small quantity of the peritoneal liquid (previously incubated for 36 hours) by means of a sterile capillary pipette, and kept at this temperature for 20 minutes. It was then rapidly cooled and placed in an incubator at 37° C.

Bacillus of Braxy.

In the case of the bouillon tubes inoculated with the peritoneal liquid obtained from a sheep which had died from this disease, the medium was found to be muddy-looking after 12 hours, and a copious evolution of gas was evidenced by the collection of gas-bells between the oil and the bouillon. After 24 hours' incubation the growth was being deposited as small floccules on the bottom and sides of the tube. At the end of 36 hours a fairly large greyish-white deposit was found at the bottom of the tube, the supernatant fluid being clear, and now giving a marked acid reaction to litmus.

The microscopical examination of the deposit revealed the presence in pure culture of a bacillus possessing the following morphological characters. In the fresh condition, the bacillus was a fairly long, thin, delicate straight rod, with rounded ends, and averaging $4.4\ \mu$ by $1.0\ \mu$. The bacilli from the bouillon deposit were usually found to be in clumps. Within the peritoneal liquid, and usually in the first culture of it, the organism was only very feebly motile, if motile at all. A few sporing forms were occasionally found in this deposit; spore formation was best obtained by incubating the original peritoneal liquid or growing the bacillus on sheep's blood. The spore, which was single, was situated at the middle or extreme end of the rod, was of a brownish colour, produced a small swelling, and gave the rod a lanceolate or drumstick appearance.

The bacillus reacted to all the ordinary bacillary stains, but was negative to Gram's process. The presence of cilia was demonstrable upon culture by the methods of staining introduced by Loeffler and van Ermengem. The cilia were extremely delicate, and special care had to be taken to avoid their detachment from the body of the bacillus. The method employed was to remove a small portion of a twelve hours' old agar surface culture grown under hydrogen, and add it to a drop of sterile distilled water on a perfectly clean coverglass. This was then allowed to evaporate in the air, and was fixed, when dry, by passing it once through the flame. A few drops of van Ermengem's mordant were poured on the film and allowed to act for 30 minutes. After gentle *lavage* in distilled water, the film was placed in a 2% solution of silver nitrate for a few seconds, and afterwards transferred direct to the sodium acetate solution,

where it was moved gently to and fro for 10-15 seconds. It was now finally transferred to 2 % silver nitrate, and left until the silver bath began to turn brown, when it was removed and again washed in distilled water. To ensure a sufficiently deep staining, it was mounted in water and examined under an oil immersion lens. If the staining was not sufficiently deep, the latter process of washing in sodium acetate solution and silver nitrate was repeated. By this process, very distinctive staining of the cilia was obtained.

The cilia varied in number from 1 to 10, averaging five; all were of equal diameter, but varied in length from 10μ to 20μ . They took their origin from any part of the periphery of the bacillus, from which they pursued a waving tortuous course. Sporing forms of the rods also possessed cilia, a fact which was in accordance with the slight movement which these also showed. As stained by Loeffler's method the cilia were hardly recognisable even with a magnification of 960 diameters on account of their extreme delicacy.

Growth on Gelatine.

In stab cultures under oil, growth was apparent in from 3-10 days—the longer the time the bacillus had been under cultivation the quicker the growth. In many cases the gelatine tubes were inoculated by means of a capillary pipette, and its track was shown by a liquefied cone of the medium demarcated from the solid part by numerous small, pin-point masses of a greyish-white colour. From the surface of the cone, horizontal, thread-like processes pursued a tortuous course to the periphery of the tube. At first, these processes were short and fluffy, and better seen at certain points of the cone. After further incubation, they increased in length and number. They might become so numerous that, matted together, they almost occluded the central cone. Here and there, throughout the growth, small, pin-point, fluffy dots were seen. Occasionally some of these processes behaved like the first inoculation, and repeated the original picture on a very small scale. The picture of a growth from a bacillus which had been cultivated in the laboratory for some time was not so complex. The liquefied cone sometimes contained no deposit, and the hair-like processes, although numerous, long and wavy, were still discrete. After longer incubation, the threads dissolved,

the central cone stood bare, and the fluffy dots were well picked out in the medium, which had become dusky-looking, and slightly more opaque. Usually only a very few gas bells developed.

Grown aërobically on surface culture, after a long incubation of weeks, the bacillus caused a slight liquefaction of the gelatine and a greyish filmy deposit.

Growth on Agar.

On several occasions the bacillus was isolated by plating out in the ordinary manner, and growing under hydrogen at 37° C. After three days, the typical colony had the following appearances:—It was about the size of a large pin-head, rounded in shape, with sharply-defined margin, and had produced a faint, bluish-white opalescence, of equal density throughout the colony. Microscopically, a small colony consisted of a large number of small dots, whilst a larger colony consisted of an agglomeration of these smaller colonies.

In stab culture a very copious evolution of gas had torn up the medium so that nothing characteristic except a greyish line along the track of inoculation was observed.

A surface culture, grown anaërobically after 24 hours, showed roundish, small, discrete colonies, which, later, ran together and spread themselves out as a fine film over the surface.

Bacillus of Louping-ill.

Growth on Bouillon.

After 12 hours' incubation, there was marked turbidity of the medium with copious evolution of gas collecting as a fine foam beneath the oil. After 48 hours' incubation, the medium became clear and distinctly acid in reaction, and the growth was deposited at the bottom of the tube in the form of a greyish-white mass. Examination in the fresh condition at varying periods of the incubation revealed the presence of a large rod with blunted ends, and broad in proportion to its length. The bacillus was only faintly motile, and no spores were visible until the incubation had been carried out for 36 hours, at which period a single small, centrally placed, refractile body had become apparent, which, later, increased in size producing an oval swelling in the centre of the bacillus.

It stained readily with the usual stains, and gave a positive reaction with Gram's process. One peculiarity with this stain was that in some cases a part or the whole of the rod did not give a deep blue stain, but was coloured a more or less faint lilac. Whether this was due to a degeneration or not could hardly be determined, since similar films stained in a normal manner with methylene blue or fuchsin. Repeated attempts with cilia stains gave in each case a negative result.

Growth on Gelatine.

Under aërobic conditions no surface growth was obtained.

Stab cultures under oil, after three days' incubation, began to show a faint greyish growth along the needle track, which, after a week's incubation, had produced a cone-shaped area of liquefaction, resembling that of the Finkler-Prior vibrio, the growth falling as a white film to the bottom of the cavity.

Radiating from the sides of this cavity were a few white processes, which divided, dichotomously, each to terminate in a small, round colony. A few gas bells developed within the liquefied area, as well as in the neighbouring solid gelatine.

Growth on Agar.

After 24 hours' incubation at 37° C., a line of greyish growth developed in stab culture, and a few gas bells had formed. After three days, this line had thrown out coarse lateral projections, but the excessive development of gas had torn up the medium.

Surface Culture grown under Hydrogen.

After two days' incubation, growth made itself apparent by the development of small, pin-head colonies, circular in outline, with sharply-defined margins. Later on these increased in size, fusing to form a well-marked white line, elevated above the level of the medium. After five days' incubation, the growth spread, laterally giving more the appearance of a cluster of grapes. The density of colour produced by the development of the colonies lay between that of a staphylococcus albus and a micrococcus candicans.

Bacillus of Disease "A."

The examination of the peritoneal liquid of certain sheep said to have died of Braxy showed the presence, not of the bacilli of Braxy or of Louping-ill, but of bacilli which have been temporarily classified by Professor Hamilton into two groups—"A" and "B" (*cf.* Report, Pt. II., p. 259).

The bacillus having been isolated by heating in glucose-bouillon, growth was obtained after 24 hours' incubation, with evolution of gas and turbidity of the medium. In three days, the medium became clear, and of an acid reaction, with a greyish-white deposit at the bottom of the tube. This deposit was, in great part, composed of coarse, straight rods, with rounded ends, varying in length from 2·8 to 6 μ , and broad in proportion to their length. Movement, if any, was very slight. They stained readily with fuchsin and methylene blue, and gave a positive reaction to Gram's process. Spores were not developed till the second day of incubation, when a single, small, rounded, refractile body was present towards one end of the rod. Cilia could not be demonstrated.

Growth on Gelatine.

Streak cultures, under aerobic conditions, showed no growth, even after weeks of incubation.

Stab cultures, grown under oil, after 7-10 days' incubation, began to show slight liquefaction of the gelatine in the form of a cone at the top of the tube, but, deeper down, tended to spread laterally. The quantity of growth in each case was very slight, amounting to little more than a faint yellow film on the sides of the cavity. Lateral projections, as in the case of Braxy and Louping-ill, were not observed.

Growth on Agar.

Streak cultures showed no change when incubated in the presence of oxygen, but, under hydrogen, after two days, a narrow line of growth had developed, made up of single, rounded, faintly greyish-tinged colonies. On the fifth day it assumed the form of a band, about $\frac{1}{4}$ in. broad, whose centre was homogeneous, but whose border was crenated from the presence of rounded colonies. The growth was very slightly

elevated above the surface of the agar, and had a moist, brightly reflecting appearance.

Stab cultures under oil developed a faint greyish streak along the line of puncture after 24 hours. This was replaced on the third day by a coarse band, with short, coarse, spiny projections, but the development of gas broke up the medium.

Bacillus of Disease "B."

After 24 hours' incubation, the glucose-bouillon was muddy-looking, and there was a marked evolution of gas. In 36 hours the medium in the upper part of the tube began to clear, while the remainder was as milky-looking as before, if not more so. After two days, the upper half of the medium was clear, and the whole of it became relatively transparent in about three days. If, at any time, the tube was but slightly shaken, the transparency of the medium was almost immediately changed to a diffuse milky opacity. The deposit was never seen on one side of the tube only, nor did it ever fall down in floccules as in braxy. A copious grey homogeneous mass was ultimately found in the bottom of the tube. The reaction of the medium became acid. The organism present was a thin, straight, scarcely motile, rod with rounded ends. Thread forms were common, and hence there was a great difference in the lengths, which varied from 2.8μ to 48.6μ . The bacilli—obtained from artificial media—were never found in clumps as was the case with braxy, but in the œdematous fluid from a guinea-pig—dead 30 hours after inoculation—huge aggregated clumps of bacilli were present. The bacillus was stained by all the ordinary stains and by Gram's process. The most favourable medium for the investigation of the sporing phase was the original peritoneal liquid, sporing being very slight on artificial media. The spore was single, relatively large, usually polar, and sometimes free.

Growth on Gelatine.

Slope cultures under aerobic conditions showed slight liquefaction and growth, only after several weeks' incubation at 21°C .

Stab cultures under oil, after two weeks, exhibited liquefaction of the medium as a narrow cylinder, lying in which were, at several places, small,

roundish, sharply demarcated, greyish-white colonies. Very delicate hair-like processes were given off from the sides of the cylinder, but in no case were fluffy dots seen amongst these as in the case of braxy, in which the processes were coarser. A few gas bells developed in the medium.

Growth on Agar.

Stab cultures under oil showed on the first day a greyish-white streak along the track of inoculation. The evolution of gas seemed to be more abundant, and to take place more rapidly than was the case with braxy, so that the medium became torn up and the character of the growth destroyed.

INDEX

- Administrative aspects of tuberculosis, 77
 Agglutination in croupous pneumonia, 393
 Albumen-metaphosphate, effect on leucocytes, 358
 Aleurone, effect on leucocytes, 357
 Aleurone, effect on opsonic index, 358
 Alformant lamp, disinfecting value, 245
 Alimentary canal as a source of infection, 3
 Anæmia, probable intestinal origin of, 37
 Appendix vermiformis, Bilharzia ova in, 48
 Auricular fibrillation, 97
 Autolysed staphylococci, effect on leucocytes, 346
 Autolysed staphylococci, effect on opsonic index, 346
- Bacillus choreæ paralyticæ, 13
 Bacillus of braxy, characters of, 404
 Bacillus of braxy, growth on agar, 406
 Bacillus of braxy, growth on gelatine, 405
 Bacillus of disease "A" cultures, 408
 Bacillus of disease "B" cultures, 409
 Bacillus of louping-ill, growth on agar, 407
 Bacillus of louping-ill, growth on bouillon, 406
 Bacillus of louping-ill, growth on gelatine, 407
 Bacillus of louping-ill, 14
 Bacillus of louping-ill, inoculation in sheep, 18
 Bacillus of louping-ill, toxins of, 27
 Bacillus pyocyaneus, autolysed cultures, 167
 Bacillus pyocyaneus, exaltation, 168
 Bacillus pyocyaneus, filtration experiments, 163
 Bilharziosis, case of, 41
 Bilharzial lesions of intestine, 47
 Bilharzial ova in appendix vermiformis, 48
 Bilharzial ova in pancreas, 43
 Bilharzial polyposis of large intestine, 51
 Bouillon, effect on leucocytes, 345
 Bouillon, effect on opsonic index, 345
- Bulbus cordis in Xiphium, 59
 Bulbus cordis malformations, 57
- Carbamic acid in urine, 270
 Cell necrosis produced by bacteria, 287
 Chorea, intestinal origin of, 36
 Cirrhosis of liver, intestinal origin of, 37
 Contagious diseases of sheep, 4
 Contagious diseases in sheep, immunity, 23
 Contagious diseases of sheep, periodicity, 5
 Contagious diseases of sheep, cause of periodicity, 25
 Contagious diseases of sheep, geographical areas affected, 5
 Cyllin, disinfecting value of, 255, 258
- Disinfection by corrosive sublimate, 260
 Disinfection by cyllin, 260
 Disinfection by formaline, 240, 257
 Disinfection by izal, 261
 Disinfection by phenol, 254, 257
 Disinfection by sanitas, 261
 Disinfection by sapo-cresol, 262
- Eck's fistula, 265
 Eck's fistula, literature on, 267
 Epidemic plague, rat theory of, 215
 Epignathus, 177
 Epignathus, case of, 178
 Epignathus, origin of, 193
 Epignathus, relations of, 190
 Epignathus, structure of, 189
 Epilepsy, intestinal origin of, 36
 Epizootic plague in Hong-Kong, 224
 Exophthalmic goitre, pathology of, 199
 Exophthalmic goitre, thymus gland in, 205
 Exophthalmic goitre, thyroid gland in, 207
 Experiments on plague toxin, 122
- Fixateur, action of heat on, 311
 Fixateur, relation to opsonin, 310
 Formaldehyde, disinfecting value, 240

- Guinea-pig, leucocytes of, 333
 Guinea-pig, physiological variations in blood, 333
- Heart, paroxysmal irregularity, 97
 Heart of *Xiphias gladius*, 58
 Hong-Kong, plague epidemics in, 223
 Hong-Kong, plague epizootics in, 223
 Hong-Kong, relation of epizootic to epidemic plague, 224
- Immunity against bacillus pyocyaneus, 157
 Immunity against plague, 113
 Immunity in pneumococcal infections, 365
 Intestine, Bilharzia lesions of, 47
 Intestinal origin of contagious diseases in sheep, 23
 Izal, disinfecting value of, 261
- Large intestine, Bilharzial polyposis, 51
 Leucocytosis and opsonic content of serum, 321
 Leucocyte in relation to opsonin, 384
 Lesser curvature of heart, 69
 Lingner's disinfecting apparatus, 241
 Liver in Bilharziosis, 42
 Louping-ill, 6
 Louping-ill, Bacillus of, 13
 Louping-ill, experimental, 18
 Louping-ill, morbid anatomy, 11
 Louping-ill, occurrence, 6
 Louping-ill, peritoneal liquid, 13
 Louping-ill, symptoms, 6
- Maladie pyocyannique, 159
 Malformations of bulbus cordis, 57
 Mammalian heart, evolution of, 61
 Metchnikoff on micro- and macrophages, 304
- Nuclein, effect on leucocytes, 350
 Nuclein, effect on opsonic index, 350
- Opsonic index, fluctuations in health, 334
 Opsonic index, variations in pneumococcal infections, 375
 Opsonin, action of heat upon, 313, 317, 384
 Opsonin, effect of diluting, 386
 Opsonin, effect of preserving, 381
 Opsonin, effect of salt upon, 387
 Opsonin, relation to leucocytosis, 391
 Opsonin, specificity of, 380
- Papayotin-albumose, effect on leucocytes, 357
- Phagocytosis during hyperleucocytosis, 359
 Phagocytosis of red corpuscles, 301
 Pilocarpine, effect on leucocytes, 347
 Pilocarpine, effect on opsonic index, 347
 Plague epidemics, rats in, 215
 Plague epidemics, bridging of, 232
 Plague antitoxin, 130
 Plague bacteriolysin, 140
 Plague immunity, 113
 Plague toxins, 114, 122
 Pneumococcus, infections, immunity in, 365
 Pneumococcus, mechanism of immunity, 370
 Pneumococcus, infections, opsonic index in, 375
 Pneumonia, agglutination in, 393
 Pneumonia, opsonic variation in, 375
 Pulmonary artery congenital stenosis, 63
 Pyocyanic immunity, 157
 Pyocyanic serum, properties, 172
- Rabbit, normal blood of, 329
 Rabbit, leucocytes of, 330
 Rats and plague, 215
 Rats, epizootics in, 223
 Red blood corpuscles, phagocytosis of, 301
 Red blood corpuscles, hæmolysis of, 301
 Right ventricle, division of, 62
- Sanitas, disinfecting value of, 261
 Sapo-Cresol, disinfecting value of, 262
 Savtchenko's experiments on phagocytosis, 309
 Sheep, contagious diseases of, 4
 Sodium cinnamate, effect on leucocytes, 335
 Sodium cinnamate, effect on opsonic index, 336
 Stenosis, congenital, of pulmonary artery, 63
- Tallianine, effect on leucocytes, 340
 Tallianine, effect on opsonic index, 341
 Tetanus, probable intestinal origin of, 35
 Thymus gland in exophthalmic goitre, 205
 Thyroid gland in exophthalmic goitre, 207
 Toxin of bacillus of louping-ill, 27
 Toxins of plague bacillus, literature, 114
 Toxins of plague bacillus, experimental, 122
 Toxicity of pyocyanic filtrates, 163
 Toxicity of pyocyanic cultures, 164
 Tuberculosis, administrative aspects, 77
 Tuberculosis, death-rate in Scotland, 79
 Tuberculosis, disinfection, 85
 Tuberculosis, isolation, 87

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